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(54) **Human antibodies specific for human transforming growth factor beta-1 and beta-2**

(57)   Human antibodies specific for human transforming growth factor-β (TGF-β), bind to TGF-β1 and/or
TGF-β2 preferentially compared with TGF-β3 and are useful in the treatment of fibrotic and
immune/inflammatory disease. A specifically disclosed antibody binds the active form of TGF-β2, neutralising
its activity but does not bind the latent form.

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At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

Figure 1(a)(i)

```
              10          20          30       40
CAG GTG CAA CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG
 Q   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R>

  50          60          70          80       90
TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTC AGT AGC TAT
 S   L   R   L   S   C   A   A   S   G   F   T   F   S   S   Y>

 100         110         120         130      140
GGC ATG CAC TGG GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG
 G   M   H   W   V   R   Q   A   P   G   K   G   L   E   W   V>

 150         160         170         180      190
GCA GTT ATA TCA TAT GAT GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG
 A   V   I   S   Y   D   G   S   N   K   Y   Y   A   D   S   V>

 200         210         220         230      240
AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT
 K   G   R   F   T   I   S   R   D   N   S   K   N   T   L   Y>

 250         260         270      280
CTG CAA ATG AAC AGC CTG AGA GCT GAG GAC ACG GCT GTG TAT TAC TGT
 L   Q   M   N   S   L   R   A   E   D   T   A   V   Y   Y   C>

 290         300         310         320      330
GCG AAA ACT GGG GAA TAT AGT GGC TAC GAT TCT AGT GGT GTG GAC GTC
 A   K   T   G   E   Y   S   G   Y   D   S   S   G   V   D   V>

 340         350         360
TGG GGC AAA GGG ACC ACG GTC ACC GTC TCC TCA
 W   G   K   G   T   T   V   T   V   S   S
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Figure 1(a)(ii)


```
           10           20           30           40
CAG GTG CAG CTG GTG CAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG
 Q   V   Q   L   V   Q   S   G   G   G   V   V   Q   P   G   R >

 50           60           70           80           90
TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTC AGT AGC TAT
 S   L   R   L   S   C   A   A   S   G   F   T   F   S   S   Y >

100          110          120          130          140
GGC ATG CAC TGG GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG
 G   M   H   W   V   R   Q   A   P   G   K   G   L   E   W   V >

150          160          170          180          190
GCA GTT ATA TCA TAT GAT GGA AGT ATT AAA TAC TAT GCA GAC TCC GTG
 A   V   I   S   Y   D   G   S   I   K   Y   Y   A   D   S   V >

200          210          220          230          240
AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT
 K   G   R   F   T   I   S   R   D   N   S   K   N   T   L   Y >

250          260          270          280
CTG CAA ATG AAC AGC CTG AGA GCT GAG GAC ACG GCT GTG TAT TAC TGT
 L   Q   M   N   S   L   R   A   E   D   T   A   V   Y   Y   C >

290          300          310          320          330
GCG CGA ACT GGT GAA TAT AGT GGC TAC GAT AGT GGT ACG GTG GAG CTC
 A   R   T   G   E   Y   S   G   Y   D   S   G   T   V   E   L >

340          350          360
TGG GGG CAA GGG ACC ACG GTC TCC TCA
 W   G   Q   G   T   T   V   S   S
```

Figure 1(a)(iii)

```
                10              20              30          40
    GAC ATC GTG ATG ACC CAG TCT CCT TCC ACC CTG TCT GCA TCT GTA GGA
     D   I   V   M   T   Q   S   P   S   T   L   S   A   S   V   G>

    50              60              70          80          90
    GAC AGA GTC ACC ATC ACT TGC CGG GCC AGT CAG GGT ATT AGT AGC TGG
     D   R   V   T   I   T   C   R   A   S   Q   G   I   S   S   W>

    100             110             120         130         140
    TTG GCC TGG TAT CAG CAG AAA CCA GGG AGA GCC CCT AAG GTC TTG ATC
     L   A   W   Y   Q   Q   K   P   G   R   A   P   K   V   L   I>

    150             160             170         180         190
    TAT AAG GCA TCT ACT TTA GAA AGT GGG GTC CCA TCA AGG TTC AGC GGC
     Y   K   A   S   T   L   E   S   G   V   P   S   R   F   S   G>

    200             210             220         230         240
    AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC AGT CTG CAA CCT
     S   G   S   G   T   D   F   T   L   T   I   S   S   L   Q   P>

    250             260             270         280
    GAA GAT TTT GCA ACT TAC TAC TGT CAA CAG AGT TAC AGT ACC CCG TGG
     E   D   F   A   T   Y   Y   C   Q   Q   S   Y   S   T   P   W>

    290             300             310         320
    ACG TTC GGC CAA GGG ACC AAG CTG GAG ATC AAA CGT
     T   F   G   Q   G   T   K   L   E   I   K   R
```

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Figure 1(b)(i)

```
          10              20              30              40
GAC ATC GTG ATG ACC CAG TCT CCA GAC TCC CTG GCT GTG TCT CTG GGC
 D   I   V   M   T   Q   S   P   D   S   L   A   V   S   L   G>

  50              60              70              80              90
GAG AGG GCC ACC ATC AAC TGC AAG TCC AGC CAG AGT CTT TTA TAC AGC
 E   R   A   T   I   N   C   K   S   S   Q   S   L   L   Y   S>

 100             110             120             130         140
TAC AAC AAG ATG AAC TAC TTA GCT TGG TAC CAG CAG AAA CCA GGA CAG
 Y   N   K   M   N   Y   L   A   W   Y   Q   Q   K   P   G   Q>

 150             160             170             180         190
CCT CCT AAG CTG CTC ATT AAC TGG GCA TCT ACC CGG GAA TCC GGG GTC
 P   P   K   L   L   I   N   W   A   S   T   R   E   S   G   V>

 200             210             220             230         240
CCT GAC CGA TTC AGT GGC AGC GGG TCT GGG ACA GAT TTC ACT CTC ACC
 P   D   R   F   S   G   S   G   S   G   T   D   F   T   L   T>

 250             260             270             280
ATC AGC AGC CTG CAG GCT GAA GAT GTG GCA GTT TAT TAC TGT CAG CAA
 I   S   S   L   Q   A   E   D   V   A   V   Y   Y   C   Q   Q>

 290             300             310             320         330
TAT TAT GCA ACT CCT CTG ACG TTC GGC CAC GGG ACC AAG GTG GAA ATC
 Y   Y   A   T   P   L   T   F   G   H   G   T   K   V   E   I>

 340
AAA CGT
 K   R
```

Figure 1(b)(ii)

Figure 1(b)(ii)

```
                 10              20              30              40
CAC GTT ATA CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
 H   V   I   L   T   Q   D   P   A   V   S   V   A   L   G   Q>

 50              60              70              80              90
ACA GTC AGG ATC ACG TGC CAA GGA GAC AGC CTC AAA AGC TAC TAT GCA
 T   V   R   I   T   C   Q   G   D   S   L   K   S   Y   Y   A>

100             110             120             130             140
AGT TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC ATC TAT
 S   W   Y   Q   Q   K   P   G   Q   A   P   V   L   V   I   Y>

150             160             170             180             190
GGT GAA AAC AGC CGG TCC CCC TCC GGG ATC CCA GAC CGA TTC TCT GGC TCC
 G   E   N   S   R   P   S   G   I   P   D   R   F   S   G   S>

200             210             220             230             240
AGC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT GGG GCT CAG GCG GAA
 S   S   G   N   T   A   S   L   T   I   T   G   A   Q   A   E>

250             260             270             280
GAT GAA GCT GAC TAT TAC TGT AAC TCC CGG GAC AGC AGT GGT ACC CAT
 D   E   A   D   Y   Y   C   N   S   R   D   S   S   G   T   H>

290             300             310             320             330
CTA GAA GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT
 L   E   V   F   G   G   G   T   K   L   T   V   L   G
```

Figure 1(c)(i)

```
                      10                  20                  30              40
CAG GTG CAA CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG
 Q   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R>

50                  60                  70              80              90
TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA CTC ACC TTC AGT AGC TAT
 S   L   R   L   S   C   A   A   S   G   L   T   F   S   S   Y>

 100                110                 120             130             140
GAC ATG CAC TGG GTC CGC CAG CCT CCA GCC AAG GGG CTG GAG TGG GTG
 D   M   H   W   V   R   Q   P   P   A   K   G   L   E   W   V>

    150                 160             170             180         190
GCA GTT ATA TCA TAT GAT GGA AGT AGT AAA TAC TAT GCA GAC TCC GTG
 A   V   I   S   Y   D   G   S   S   K   Y   Y   A   D   S   V>

        200             210             220             230         240
AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT
 K   G   R   F   T   I   S   R   D   N   S   K   N   T   L   Y>

        250             260         270             280
CTG CAA ATG AAC AGC CTG AGA GCT GAG GAC ACG GCT GTG TAT TAC TGT
 L   Q   M   N   S   L   R   A   E   D   T   A   V   Y   Y   C>

        290             300             310         320         330
GCG CGA ACT GGT GAA TAT AGT GGC TAC GAC AGT GGT GTG GAG CTC
 A   R   T   G   E   Y   S   G   Y   D   S   G   V   E   L>

    340             350             360
TGG GGG CAA GGG ACC ACG GTC ACC GTC TCC TCA
 W   G   Q   G   T   T   V   T   V   S   S
```

Figure 2(a)(i)

```
                  10              20              30           40
GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG
 E   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R>

 50              60              70           80              90
TCC CTG AGA CTC TCC TGT GCA GCG TCT GGA TTC ACC TTC AGT AGC TAT
 S   L   R   L   S   C   A   A   S   G   F   T   F   S   S   Y>

100             110             120          130             140
GGC ATG CAC TGG GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG
 G   M   H   W   V   R   Q   A   P   G   K   G   L   E   W   V>

150             160             170          180             190
GCA GTT ATA TGG TAT GAT GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG
 A   V   I   W   Y   D   G   S   N   K   Y   Y   A   D   S   V>

200             210             220          230             240
AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT
 K   G   R   F   T   I   S   R   D   N   S   K   N   T   L   Y>

250             260             270          280
CTG CAA ATG GAC AGC CTG AGA GCC GAG GAC ACG GCC GTG TAT TAC TGT
 L   Q   M   D   S   L   R   A   E   D   T   A   V   Y   Y   C>

290             300             310          320             330
GGA AGA ACG CTG GAG TCT AGT TTG TGG GGC CAA GGC ACC CTG GTC ACC
 G   R   T   L   E   S   S   L   W   G   Q   G   T   L   V   T>

340
GTC TCC TCA
 V   S   S
```

Figure 2(a)(ii)

```
              10          20          30          40
GAG ATT CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGA
 E   I   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R>

      50          60          70          80          90
TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTC AGT AGC TAT
 S   L   R   L   S   C   A   A   S   G   F   T   F   S   S   Y>

     100         110         120         130         140
GCT ATG CAC TGG GTC CGC CAG CCA GCC AAG GGG CTG GAG TGG GTG
 A   M   H   W   V   R   Q   P   A   K   G   L   E   W   V>

     150         160         170         180         190
GCA GTT ATA TCA TAT GAT GGA AGC AAT AAA TAC TAC GCA GAC TCC GTG
 A   V   I   S   Y   D   G   S   N   K   Y   Y   A   D   S   V>

     200         210         220         230         240
AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT
 K   G   R   F   T   I   S   R   D   N   S   K   N   T   L   Y>

     250         260         270         280
CTG CAA ATG AAC AGC CTG AGA GCT GAG GAC ACG GCC GTG TAT TAC TGT
 L   Q   M   N   S   L   R   A   E   D   T   A   V   Y   Y   C>

     290         300         310         320         330
GCA AGA GCG GGG TTG GAA ACG ACG TGG GGC CAA GGA ACC CTG GTC ACC
 A   R   A   G   L   E   T   T   W   G   Q   G   T   L   V   T>

     340         350
GTC TCC TCA AGT GG
 V   S   S   S   G
```

Figure 2(b)(i)

```
          10          20          30          40
GAT GTT GTG ATG ACT CAG TCT CCA TCC CTG TCT GCA TCT GTA GGA
 D   V   V   M   T   Q   S   P   S   L   S   A   S   V   G>

 50          60          70          80          90
GAC AGA GTC ACC ATC ACT TGC CGG GCC AGT CAG GGC ATT AGC AAT TAT
 D   R   V   T   I   T   C   R   A   S   Q   G   I   S   N   Y>

100         110         120         130         140
TTA GCC TGG TAT CAG CAA AAA CCA GGG AAA GCC CCT AAG CTC CTG ATC
 L   A   W   Y   Q   Q   K   P   G   K   A   P   K   L   L   I>

150         160         170         180         190
TAT AAG GCA TCT ACT TTA GAA AGT GGG GTC CCA TCA AGG TTC AGT GGC
 Y   K   A   S   T   L   E   S   G   V   P   S   R   F   S   G>

200         210         220         230         240
AGT GGA TCT GGG ACA GAA TTC ACT CTC ACA ATC AGC AGT CTG CAA CCT
 S   G   S   G   T   E   F   T   L   T   I   S   S   L   Q   P>

250         260         270         280
GAA GAT TTT GCA ACT TAC TAC TGT CAA CAG AGT TAC AGT ACC CCT CGA
 E   D   F   A   T   Y   Y   C   Q   Q   S   Y   S   T   P   R>

290         300         310         320         330
ACG TTC GGC CAA GGG ACC AAA GTG GAT ATC AAA CGT
 T   F   G   Q   G   T   K   V   D   I   K   R
```

Figure 2(b)(ii)

```
            10              20              30              40
TCG TCT GAG CTG ACT CAG CCT GCT GTG TCT GTG GCC TTG GGA CAG
 S   S   E   L   T   Q   P   A   V   S   V   A   L   G   Q>

 50             60              70              80              90
ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA AGC TAT TAT GCA
 T   V   R   I   T   C   Q   G   D   S   L   R   S   Y   Y   A>

100             110             120             130             140
AGC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC ATC TAT
 S   W   Y   Q   Q   K   P   G   Q   A   P   V   L   V   I   Y>

150             160             170             180             190
GGT AAA AAC AAC CGG CCC TCA GGG ATC CCA GAC CGA TTC GCT GGC TCC
 G   K   N   N   R   P   S   G   I   P   D   R   F   A   G   S>

200             210             220             230             240
AAC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT GGG GCT CAG GCG GAG
 N   S   G   N   T   A   S   L   T   I   T   G   A   Q   A   E>

250             260             270             280
GAT GAG GCT GAC TAT TAC TGT AGC TCC CGG GAC AGC AGT GGT AAC CAT
 D   E   A   D   Y   Y   C   S   S   R   D   S   S   G   N   H>

290             300             310             320
GTG GTT TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT
 V   V   F   G   G   G   T   K   L   T   V   L   G>
```

Figure 2(b)(iii)

```
                      10              20              30              40
TCG TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
 S   S   E   L   T   Q   D   P   A   V   S   V   A   L   G   Q>

 50              60              70              80              90
ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA AGC TAT TAT GCA
 T   V   R   I   T   C   Q   G   D   S   L   R   S   Y   Y   A>

100             110             120             130             140
AGC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC ATC TAT
 S   W   Y   Q   Q   K   P   G   Q   A   P   V   L   V   I   Y>

150             160             170             180             190
GGT AAA AAC AAC CGG CCC TCA GGG ATC CCA GAC TTC TCT GGC TCC
 G   K   N   N   R   P   S   G   I   P   D   F   S   G   S>

200             210             220             230             240
AGC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT GGG GCT CAG GCG GAA
 S   S   G   N   T   A   S   L   T   I   T   G   A   Q   A   E>

250             260             270             280
GAT GAG GCT GAC TAT TAC TGT AAC TCC CGG GAC AGC AGT AGT ACC CAT
 D   E   A   D   Y   Y   C   N   S   R   D   S   S   S   T   H>

290             300             310             320             330
CGA GGG GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT
 R   G   V   F   G   G   G   T   K   L   T   V   L   G
```

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Figure 2(b)(iv)

```
          10                  20                  30                  40
GAA GTT GTG CTG ACT CAG TCT CCA TCC CTG TCT GCA TCT GTA GGA
 E   V   V   L   T   Q   S   P   S   L   S   A   S   V   G>

 50                  60                  70                  80                  90
GAC AGA GTC ACC ATC ACT TGC CGG GCA AGT CAG GGC ATT GGA GAT GAT
 D   R   V   T   I   T   C   R   A   S   Q   G   I   G   D   D>

100                 110                 120                 130                 140
TTG GGC TGG TAT CAG CAG AAG CCA GGG AAA GCC CCT ATC CTC CTG ATC
 L   G   W   Y   Q   Q   K   P   G   K   A   P   I   L   L   I>

150                 160                 170                 180                 190
TAT GGT ACA TCC ACT TTA CAA AGT GGG GTC CCG TCA AGG TTC AGC GGC
 Y   G   T   S   T   L   Q   S   G   V   P   S   R   F   S   G>

200                 210                 220                 230                 240
AGT GGA TCT GGC ACA GAT TTC ACT CTC ACC ATC AAC AGC CTG CAG CCT
 S   G   S   G   T   D   F   T   L   T   I   N   S   L   Q   P>

250                 260                 270                 280
GAA GAT TTT GCA ACT TAT TAC TGT CTA CAA GAT TCC AAT TAC CCG CTC
 E   D   F   A   T   Y   Y   C   L   Q   D   S   N   Y   P   L>

290                 300                 310                 320
ACT TTC GGC GGA GGG ACA CGA CTG GAG ATT AAA CGT
 T   F   G   G   G   T   R   L   E   I   K   R
```

Figure 2 (b) (v)

```
              10          20          30          40
TCG TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
 S   S   E   L   T   Q   D   P   A   V   S   V   A   L   G   Q>

50          60          70          80          90
ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA AAC TAT TAT GCA
 T   V   R   I   T   C   Q   G   D   S   L   R   N   Y   Y   A>

100         110         120         130         140
AAC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC ATC TAT
 N   W   Y   Q   Q   K   P   G   Q   A   P   V   L   V   I   Y>

150         160         170         180         190
GGT AAA AAC AAC CGG CCC TCA GGG ATC CCA GAC CGA TTC TCT GGC TCC
 G   K   N   N   R   P   S   G   I   P   D   R   F   S   G   S>

200         210         220         230         240
AGC TCA GGG AAC ACA GCT TCC TTG ACC ATC ACT GGG GCT CGG GCG GAA
 S   S   G   N   T   A   S   L   T   I   T   G   A   R   A   E>

250         260         270         280
GAT GAG GGT GTC TAT TAC TGT AAC TCC CGG GAC AGC AGT GGT GCG GTT
 D   E   G   V   Y   Y   C   N   S   R   D   S   S   G   A   V>

290         300         310         320
TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT
 F   G   G   G   T   K   L   T   V   L   G
```

Figure 3

PARENT (1-B2)	A	R	T	G	E	Y	S	G	Y	D	S	S	G	V	D	V	W
27-C1	A	R	T	G	E	Y	S	G	Y	D	T	S	G	V	E	L	W
27-D7	A	R	T	R	E	Y	S	G	H	D	S	S	G	V	D	D	W
27-E10	A	R	T	G	P	F	S	G	Y	D	S	S	G	E	D	V	R
27-H1	A	R	T	E	E	Y	S	G	Y	D	S	S	G	V	D	V	W
27-E2	A	Q	T	R	E	Y	T	G	Y	D	S	S	G	V	D	V	W
28-A11	A	R	T	E	E	Y	S	G	F	D	S	T	G	E	D	V	W
28-E12	A	R	T	E	E	F	S	G	Y	D	S	S	G	V	D	V	W
28-H10	A	R	T	G	E	Y	S	G	Y	H	S	S	G	V	D	V	R
31-G2	A	R	T	E	E	F	S	G	Y	D	S	S	G	V	D	V	W
30-B6	A	R	A	G	P	F	S	G	Y	D	S	S	G	E	D	V	R
30-E9	A	R	T	G	P	F	S	G	Y	D	S	S	G	E	D	V	W
30-F6	A	R	T	E	E	F	S	G	Y	D	S	S	G	V	D	V	W
30-D2	A	R	T	G	E	Y	S	G	Y	D	S	S	G	E	L	V	W
31-A2	A	R	T	E	E	F	S	G	Y	D	S	T	G	E	E	V	W
31-E11	A	R	T	E	E	F	S	G	Y	D	S	S	G	V	D	V	W
31-F1	A	R	T	G	E	Y	S	G	Y	D	S	S	G	E	D	V	W

Figure 4

```
            10          20          30          40
TCG TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
 S   S   E   L   T   Q   D   P   A   V   S   V   A   L   G   Q>

 50          60          70          80          90
ACA GTT AGG ATC ACT TCC CAA GGA GAC AGT CTC AGA AGC TAT TAC ACA
 T   V   R   I   T   S   Q   G   D   S   L   R   S   Y   Y   T>

100         110         120         130         140
AAC TGG TTT CAG CAG AAG CCA GGA CAG CCC CCT CTA CTT GTC GTC TAT
 N   W   F   Q   Q   K   P   G   Q   P   P   L   L   V   V   Y>

150         160         170         180         190
GCT AAA AAT AAG CGG CCC TCA GGG ATC CCA GAC CGA TTC TCT GGC TCC
 A   K   N   K   R   P   S   G   I   P   D   R   F   S   G   S>

200         210         220         230         240
AGC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT GGG GCT CAG GCG GAA
 S   S   G   N   T   A   S   L   T   I   T   G   A   Q   A   E>

250         260         270         280
GAT GAG GCT GAC TAT TAC TGT CAT TCC CGG GAC AGC AGT GGT AAC CAT
 D   E   A   D   Y   Y   C   H   S   R   D   S   S   G   N   H>

290         300         310         320
GTG CTT TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT
 V   L   F   G   G   G   T   K   L   T   V   L   G
```

Figure 5

16|38

```
          H
          i
          n
          d
          I
          I
          I
          aagcttgccgccaccatggactggacctggcgcgtgttttgcctgctcgccgtggcccct
       1  ---------+---------+---------+---------+---------+---------+  60
          ttcgaacggcggtggtacctgacctggaccgcgcacaaaacggacgagcggcaccggggga

     a    K   L   A   A   T   M   D   W   T   W   R   V   F   C   L   L   A   V   A   P   -

                                                                              B
                                                                              s
          S                               P                                   t
          f                               s                                   E
          i                               t                                   I
          I                               I                                   I
          ggggcccacagccaggtgcaactgcagcagtccggtgccaagggaccacggtcaccgtct
      61  ---------+---------+---------+---------+---------+---------+  120
          cccccgggtgtcggtccacgttgacgtcgtcaggccacggttccctggtgccagtggcaga

     a    G   A   H   S   Q   V   Q   L   Q   Q   S   G   A   K   G   P   R   S   P   S   -

                            B               E
                            a               c
                            m               o
                            H               R
                            I               I
          cctcaggtgagtggatccgaattc
     121  ---------+---------+-----  144
          ggagtccactcacctaggcttaag

     a    P   Q   V   S   G   S   E   F   -
```

Fig.6.
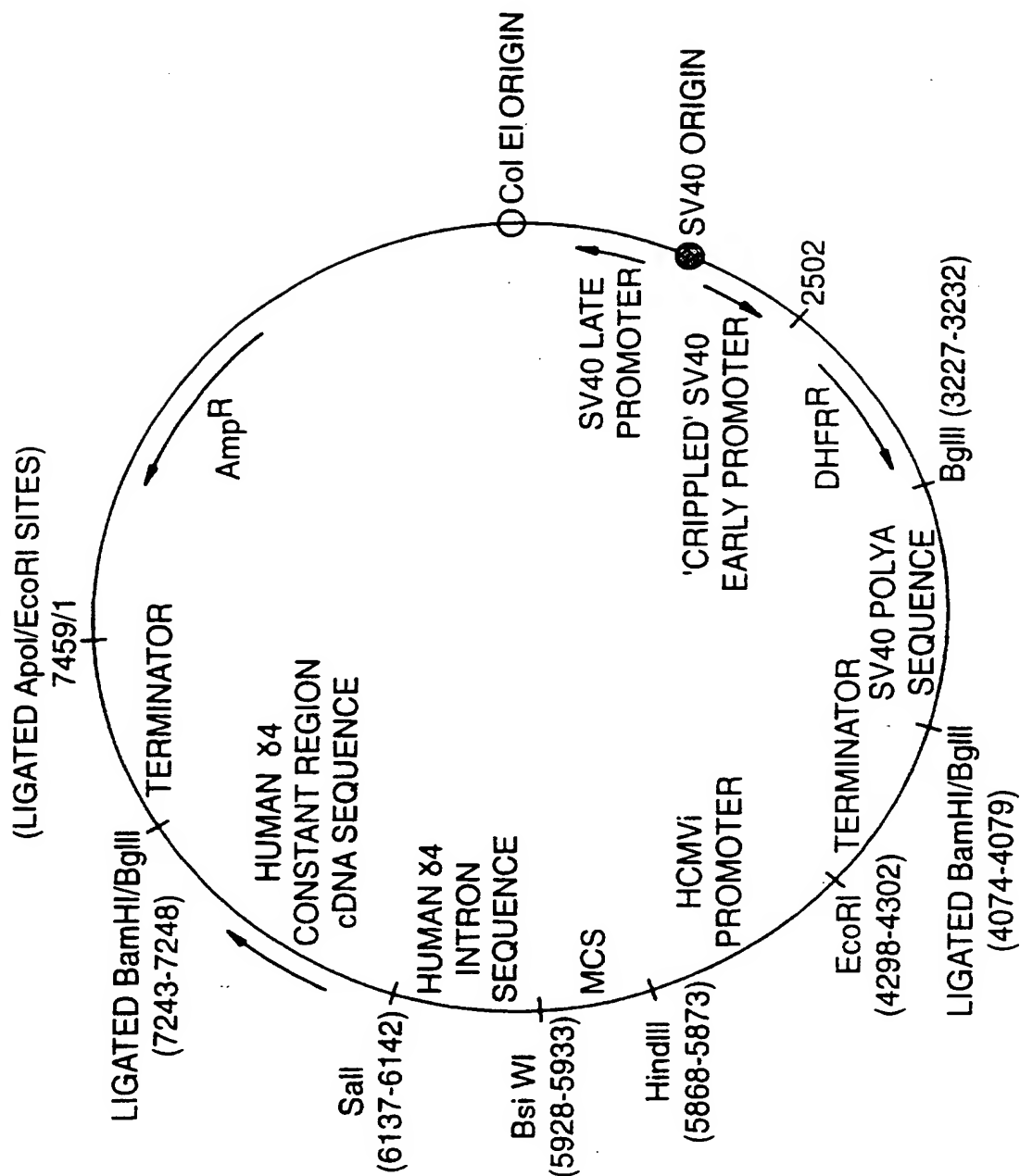
Figure 7

```
     H
     i
     n
     d
     I
     I
     I
     aagcttcgccaccatgggatggagctgtatcatcctcttcttggtagcaacagctacagg
  1  ---------+---------+---------+---------+---------+---------+  60
     ttcgaagcggtggtaccctacctcgacatagtaggagaagaaccatcgttgtcgatgtcc

         M  G  W  S  C  I  I  L  F  L  V  A  T  A  T


     taaggggctcacagtagcaggcttgaggtctggacatatatatgggtgacaatgacatcc
 61  ---------+---------+---------+---------+---------+---------+  120
     attccccgagtgtcatcgtccgaactccagacctgtatatatacccactgttactgtagg

                 A                                S
                 P                                a
                 a                                c
                 L                                I
                 I
     actttgcctttctctccacaggtgtgcactccgacattgagctcacccagtctccagaca
121  ---------+---------+---------+---------+---------+---------+  180
     tgaaacggaaagagaggtgtccacacgtgaggctgtaactcgagtgggtcagaggtctgt

             G  V  H  S  D  I  E  L

                                                            B
         X                                                  a
         h                                                  m
         o                                                  H
         I                                                  I
     aagctcgagctgaaacgtgagtagaattttaaactttgcttcctcaattggatcc
181  ---------+---------+---------+---------+---------+----  234
     ttcgagctcgactttgcactcatcttaaatttgaaacgaaggagttaacctagg

         L  E  L  K
```

Fig.8.

Fig.9.

Fig.10.

14F12
6B1
6A5
6H1
R&D

Fig.11.

Genzyme Mab

6H1

6B1

6A5
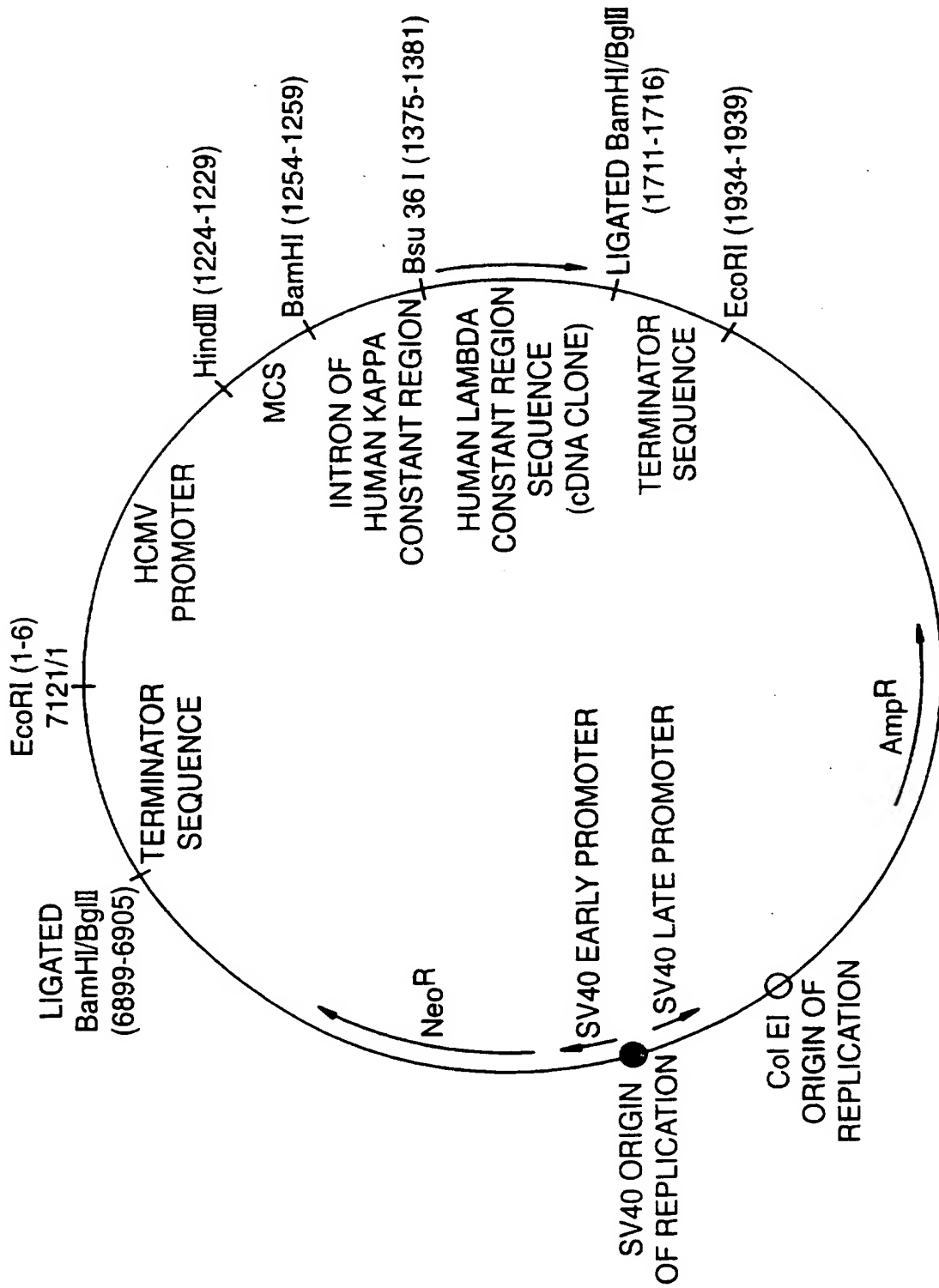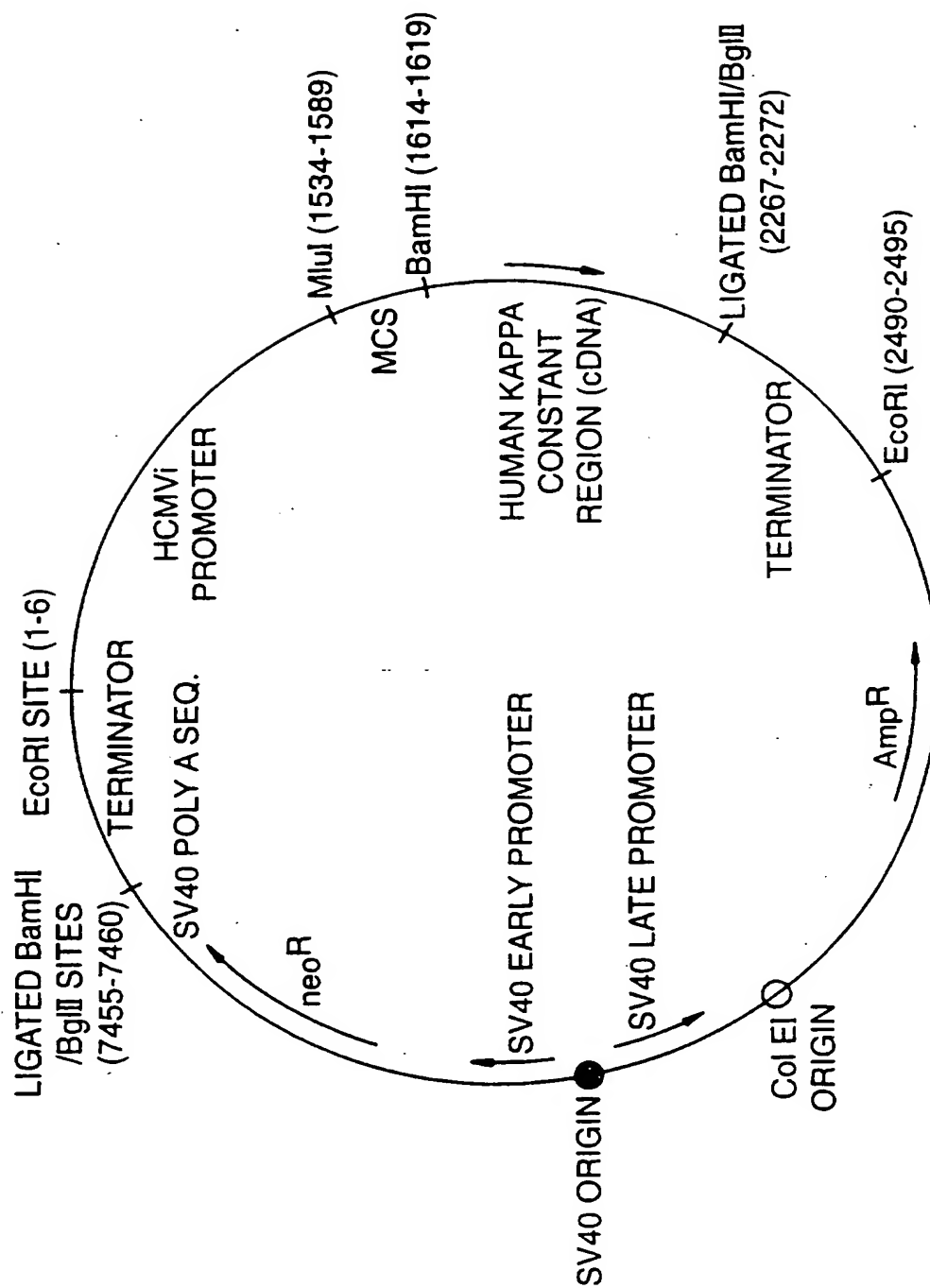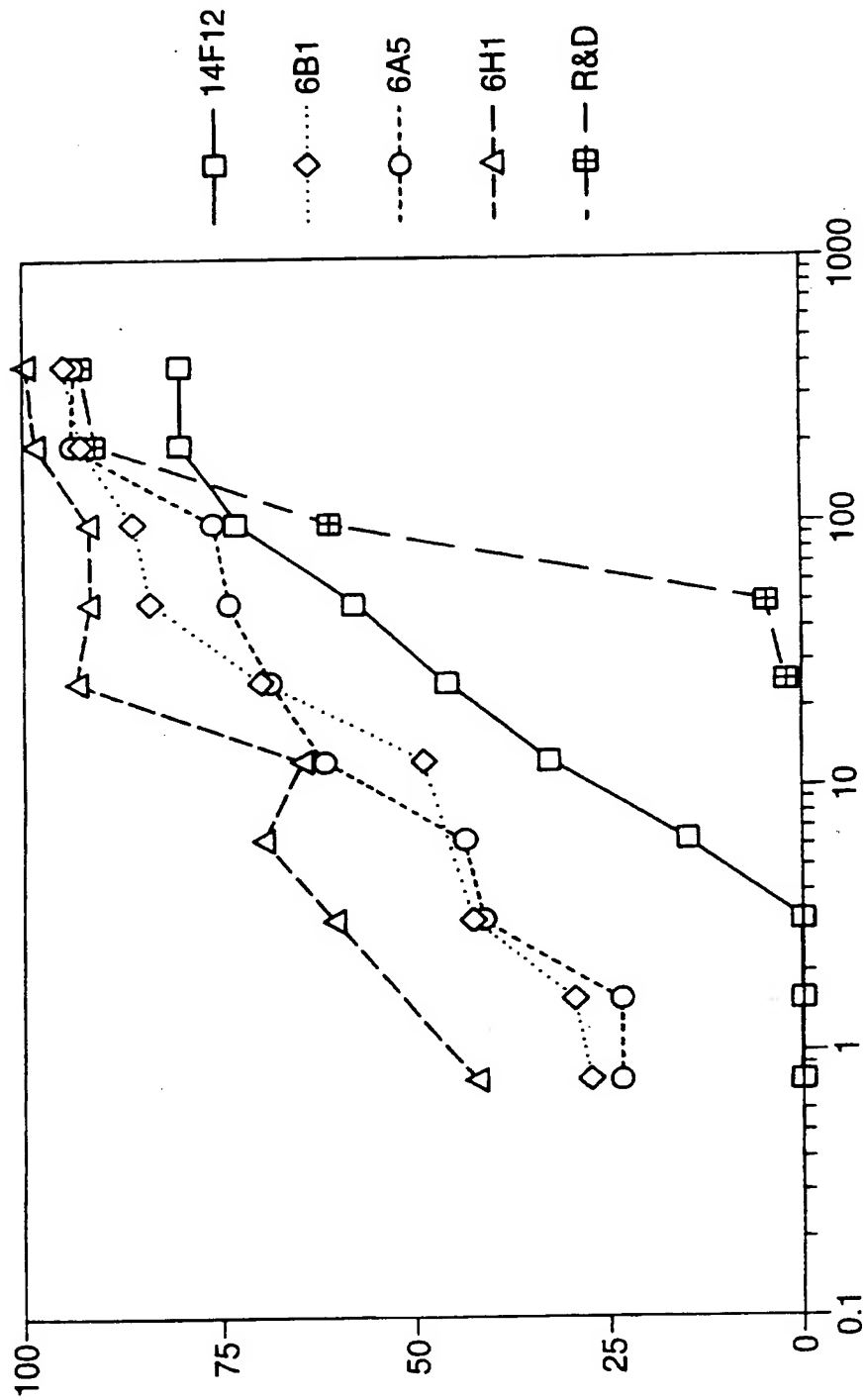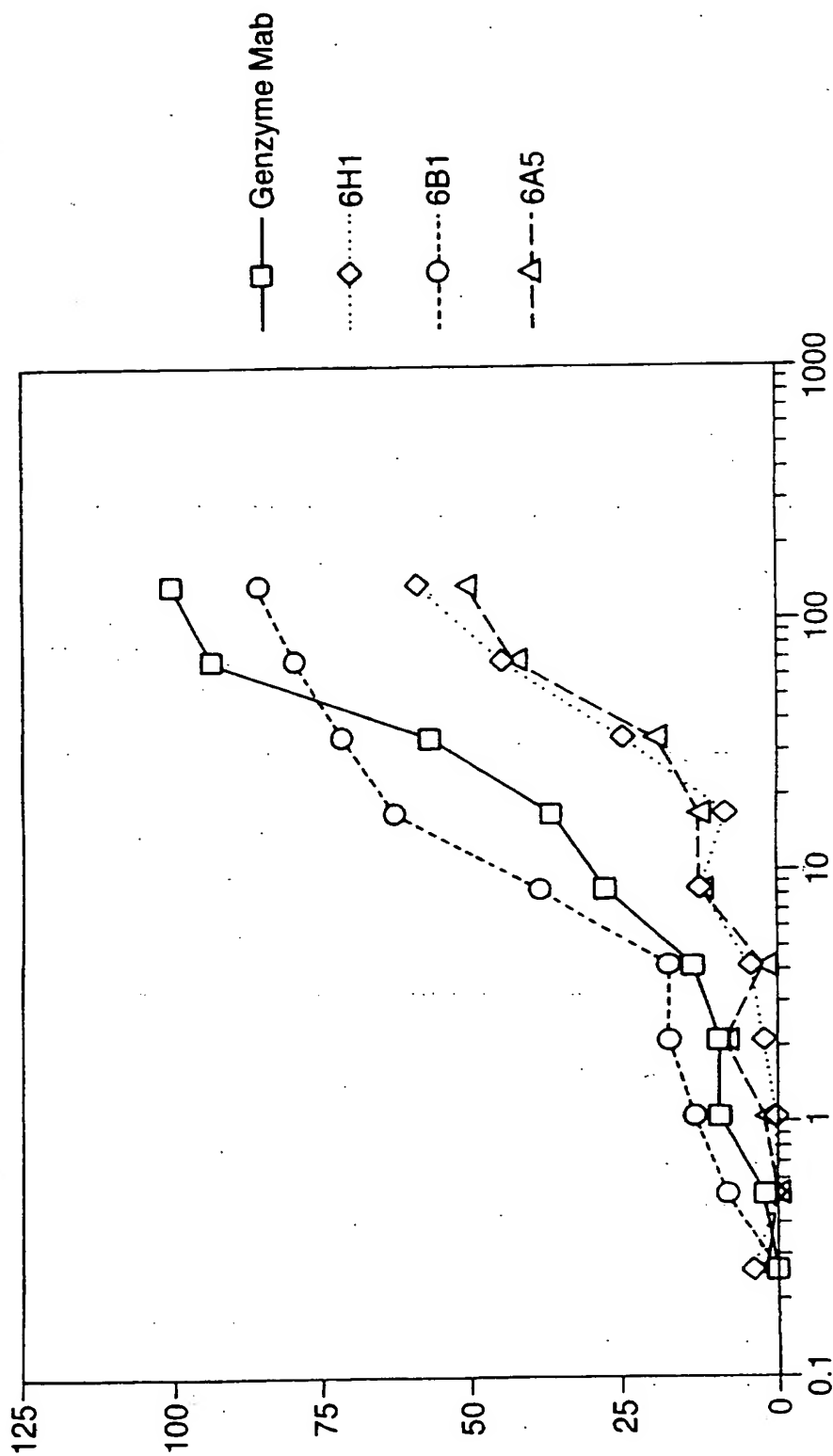
Fig.12.
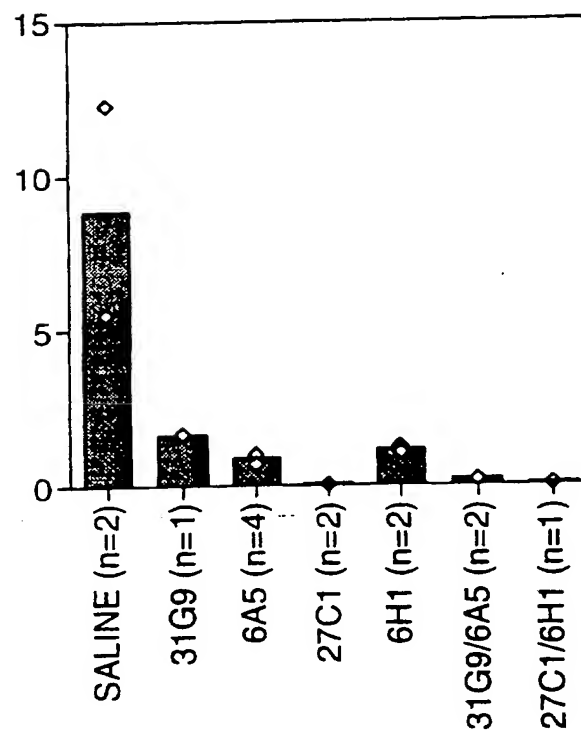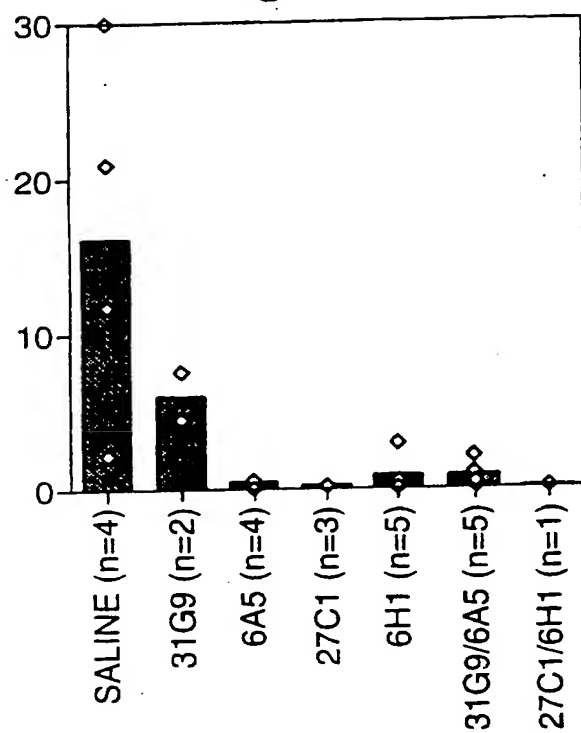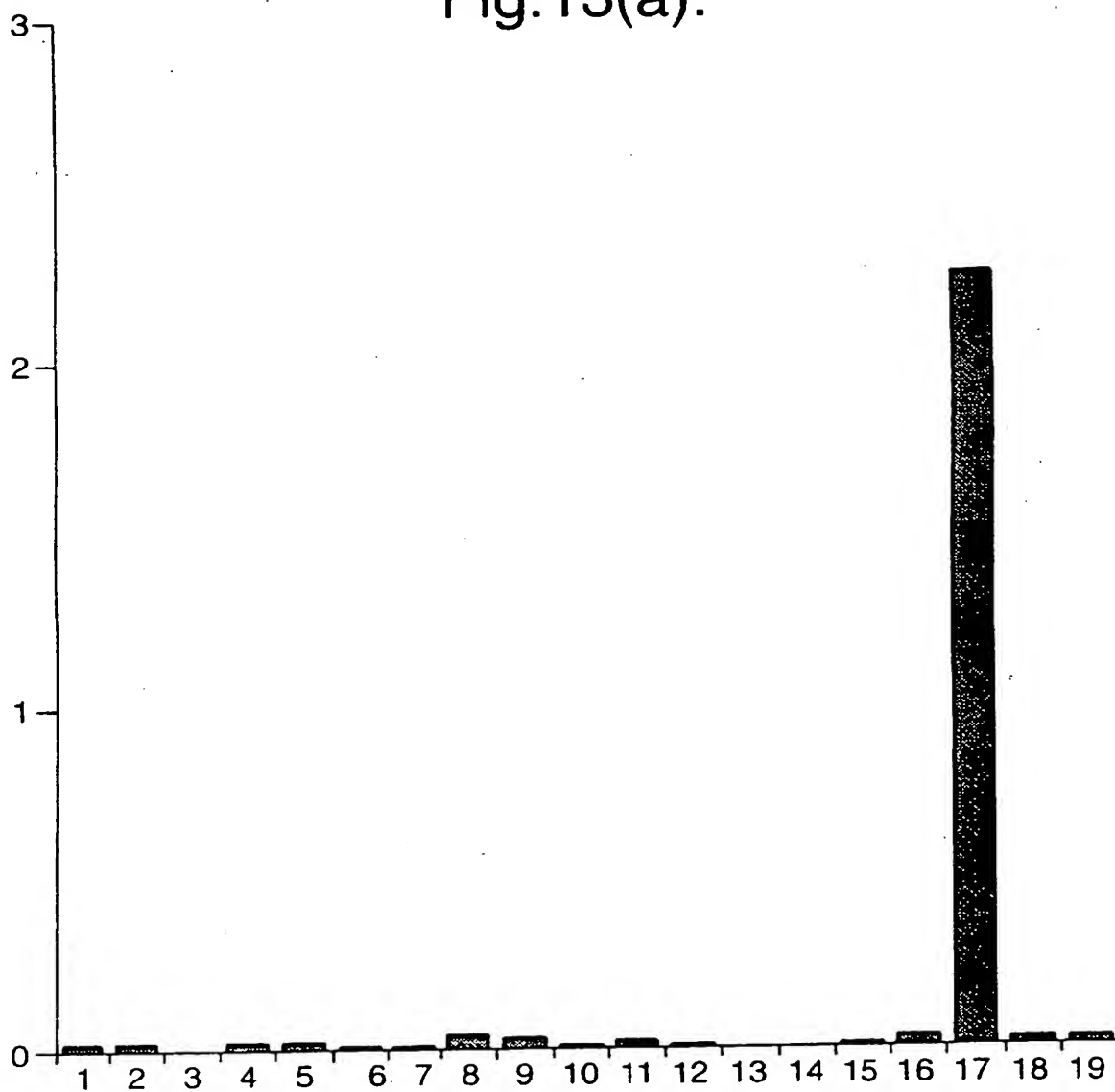
Fig.13(a).

Fig.13(b).
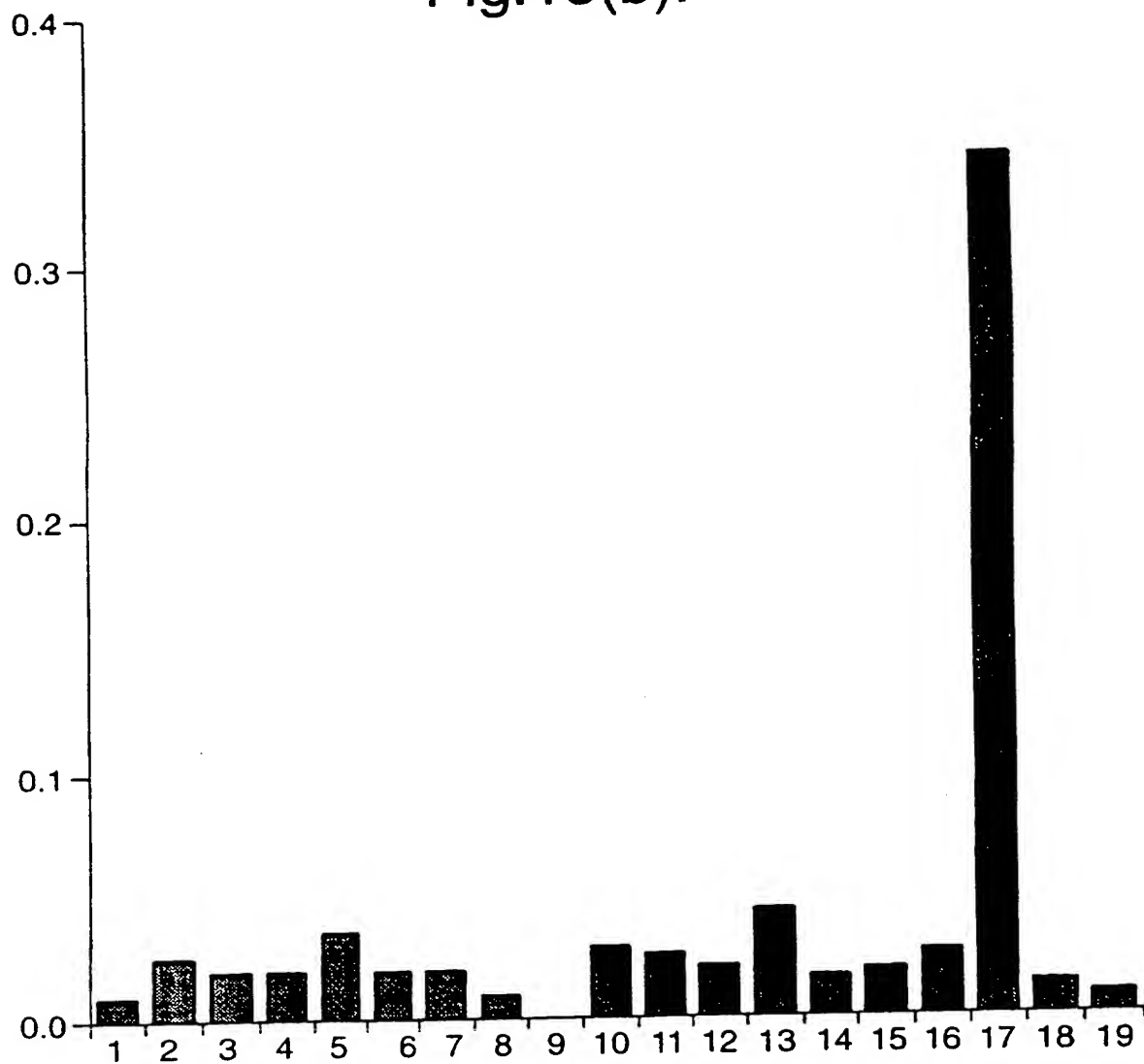
Figure 14

```
                            10                    20                    30                    40
GAA ATT GTG CTG ACT CAG TCT CCA TCC CTG TCT GCA TCT GTA GGA
 E   I   V   L   T   Q   S   P   S   L   S   A   S   V   G>

 50                   60                    70                    80                    90
GAC AGA GTC ACC ATC ACT TGC CGG GCA AGT CAG GGC ATT GGA GAT GAT
 D   R   V   T   I   T   C   R   A   S   Q   G   I   G   D   D>

100                  110                   120                   130                   140
TTG GGC TGG TAT CAG CAG AAG CCA GGG AAA GCC CCT ATC CTC CTG ATC
 L   G   W   Y   Q   Q   K   P   G   K   A   P   I   L   L   I>

            150                   160                   170                   180                   190
TAT GGT ACA TCC ACT TTA CAA AGT GGG GTC CCG TCA AGG TTC AGC GGC
 Y   G   T   S   T   L   Q   S   G   V   P   S   R   F   S   G>

            200                   210                   220                   230                   240
AGT GGA TCT GGC ACA GAT TTC ACT CTC ACC ATC AAC AGC CTG CAG CCT
 S   G   S   G   T   D   F   T   L   T   I   N   S   L   Q   P>

            250                   260                   270                   280
GAA GAT TTT GCA ACT TAT TAC TGT CTA CAA GAT TCC AAT TAC CCG CTC
 E   D   F   A   T   Y   Y   C   L   Q   D   S   N   Y   P   L>

290                  300                   310                   320
ACT TTC GGC GGA GGG ACA CGA CTG GAG ATT AAA CGT
 T   F   G   G   G   T   R   L   E   I   K   R>
```

Fig.15.

Fig.16.

── □ ── 6H1 IgG4
········◇········ Genzyme mouse Mab
── ◯ ── 6B1 IgG4

Fig.17.

- ──□── 6H1 IgG4
- ·····◇····· 6B1 IgG4
- ----O---- Genzyme mouse Mab

Fig.18.

Legend:
- —□— 6H1 IgG4
- ·····◇····· 6B1 IgG4
- ---○--- Genzyme mouse Mab
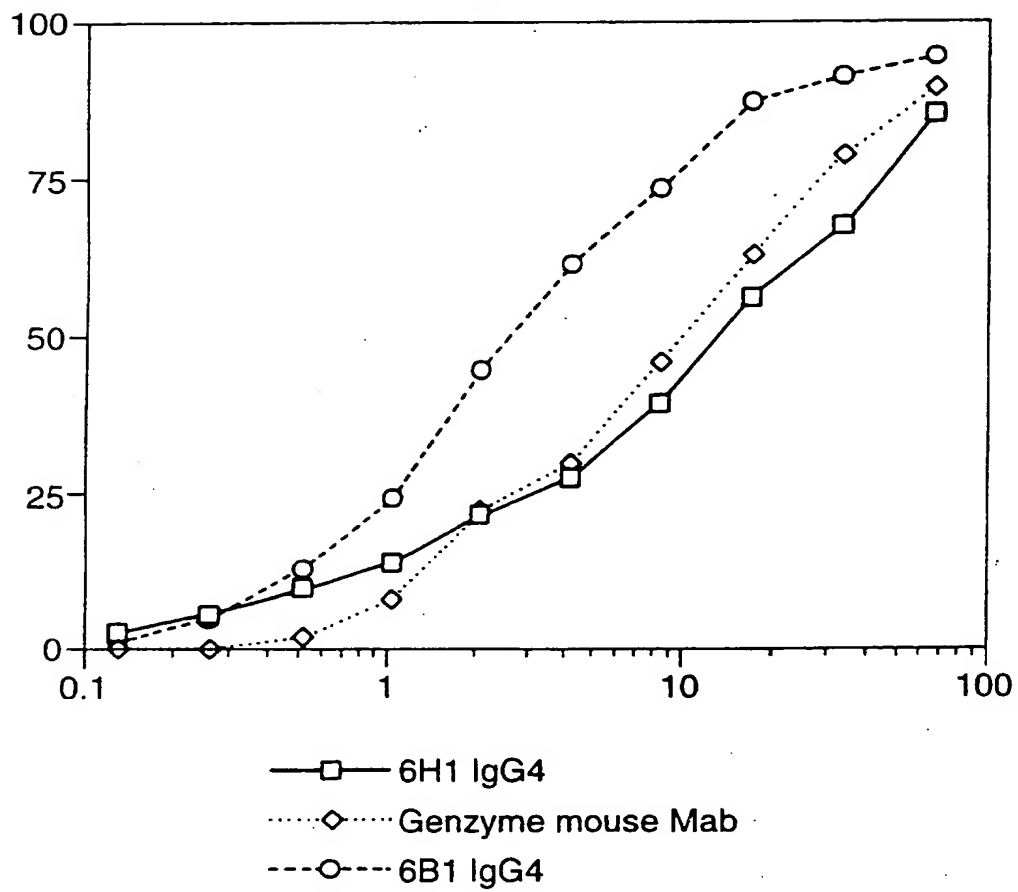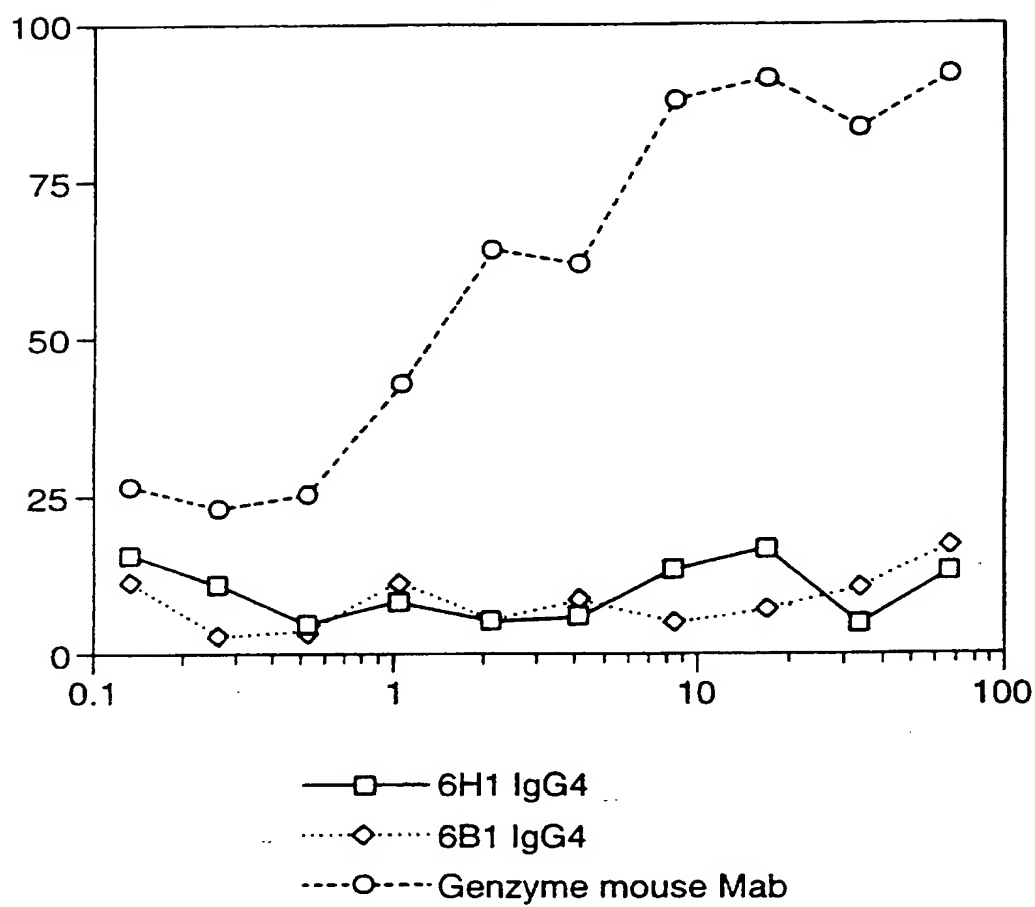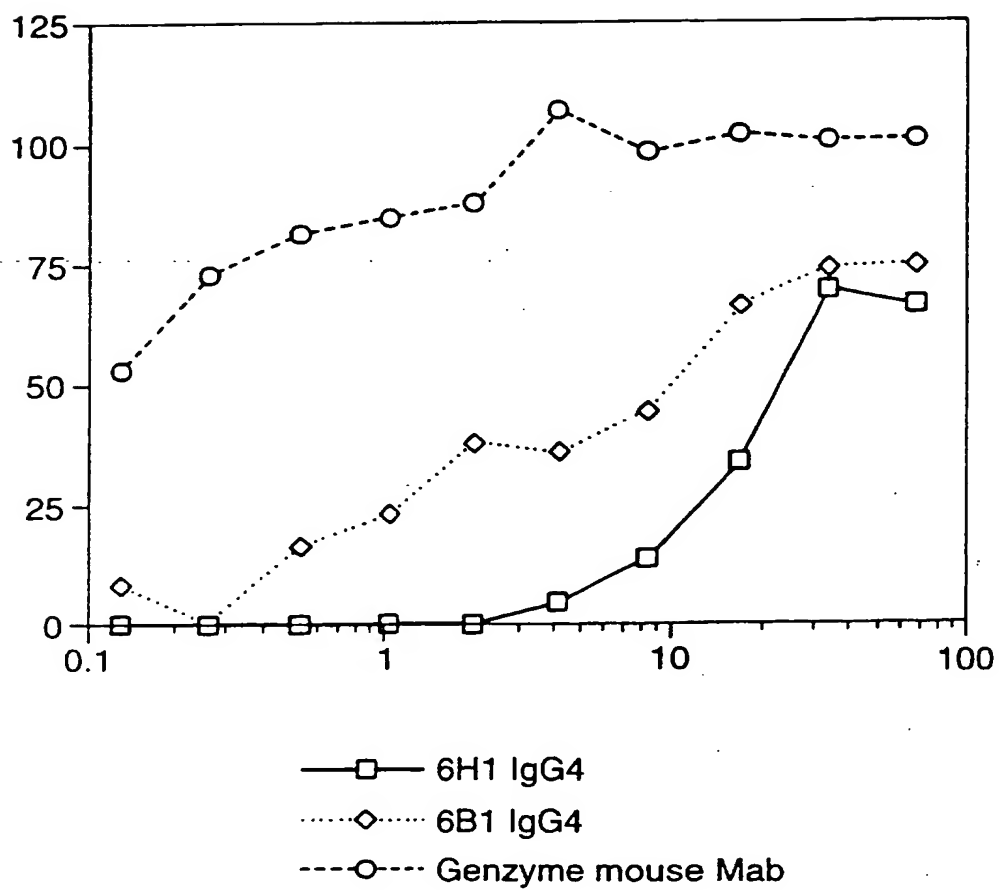
Figure 19

(i)

```
                10                  20                  30                  40
GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG
 E   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R>

50                  60                  70                  80                  90
TCC CTG AGA CTC TCC TGT GCA GCG TCT GGA TTC ACC TTC AGT AGC TAT
 S   L   R   L   S   C   A   A   S   G   F   T   F   S   S   Y>

100                 110                 120                 130                 140
GGC ATG CAC TGG GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG
 G   M   H   W   V   R   Q   A   P   G   K   G   L   E   W   V>

150                 160                 170                 180                 190
GCA GTT ATA TGG TAT GAT GGA AGT AAT AAA TAC TAT GCA·GAC TCC GTG
 A   V   I   W   Y   D   G   S   N   K   Y   Y   A   D   S   V>

200                 210                 220                 230                 240
AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT
 K   G   R   F   T   I   S   R   D   N   S   K   N   T   L   Y>

250                 260                 270                 280
CTG CAA ATG GAC AGC CTG AGA GCC GAG GAC ACG GCC GTG TAT TAC TGT
 L   Q   M   D   S   L   R   A   E   D   T   A   V   Y   Y   C>

290                 300                 310                 320                 330
GGA AGA ACG CTG GAG TCT AGT TTG TGG GGC CAA GGC ACC CTG GTC ACC
 G   R   T   L   E   S   S   L   W   G   Q   G   T   L   V   T>

340
GTC TCC TCA
 V   S   S
```

Figure 19 (ii)

```
         10              20              30              40
TCG TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
 S   S   E   L   T   Q   D   P   A   V   S   V   A   L   G   Q>

 50              60              70              80              90
ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA AGC TAT TAT GCA
 T   V   R   I   T   C   Q   G   D   S   L   R   S   Y   Y   A>

100             110             120             130             140
AGC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC ATC TAT
 S   W   Y   Q   Q   K   P   G   Q   A   P   V   L   V   I   Y>

150             160             170             180             190
GGT AAA AAC AAC CGG CCC TCA GGG ATC CCA GAC CGA TTC TCT GGC TCC
 G   K   N   N   R   P   S   G   I   P   D   R   F   S   G   S>

200             210             220             230             240
AGC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT GGG GCT CAG GCG GAA
 S   S   G   N   T   A   S   L   T   I   T   G   A   Q   A   E>

250             260             270             280
GAT GAG GCT GAC TAT TAC TGT AAC TCC CGG GAC AGC AGT AGT ACC CAT
 D   E   A   D   Y   Y   C   N   S   R   D   S   S   S   T   H>

290             300             310             320             330
CGA GGG GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT
 R   G   V   F   G   G   G   T   K   L   T   V   L   G
```

Figure 19 (iii)

```
                10              20              30              40
TCG TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG
 S   S   E   L   T   Q   D   P   A   V   S   V   A   L   G   Q>

        50              60              70              80              90
ACA GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA AGC TAT TAT GCA
 T   V   R   I   T   C   Q   G   D   S   L   R   S   Y   Y   A>

       100             110             120             130             140
AGC TGG TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC ATC TAT
 S   W   Y   Q   Q   K   P   G   Q   A   P   V   L   V   I   Y>

       150             160             170             180             190
GGT AAA AAC AAC CGG CCC TCA GGG ATC CCA GAC CGA TTC GCT GGC TCC
 G   K   N   N   R   P   S   G   I   P   D   R   F   A   G   S>

       200             210             220             230             240
AAC TCA GGA AAC ACA GCT TCC TTG ACC ATC ACT GGG GCT CAG GCG GAG
 N   S   G   N   T   A   S   L   T   I   T   G   A   Q   A   E>

       250             260             270             280
GAT GAG GCT GAC TAT TAC TGT AGC TCC CGG GAC AGC AGT GGT AAC CAT
 D   E   A   D   Y   Y   C   S   S   R   D   S   S   G   N   H>

       290             300             310             320
GTG GTT TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT
 V   V   F   G   G   G   T   K   L   T   V   L   G
```

Figure 19 (iv)

```
            10          20          30          40
GAT GTT GTG ATG ACT CAG TCT CCA TCC CTG TCT GCA TCT GTA GGA
 D   V   V   M   T   Q   S   P   S   S   L   S   A   S   V   G>

   50          60          70          80          90
GAC AGA GTC ACC ATC ACT TGC CGG GCC AGT CAG GGC ATT AGC AAT TAT
 D   R   V   T   I   T   C   R   A   S   Q   G   I   S   N   Y>

  100         110         120         130         140
TTA GCC TGG TAT CAG CAA AAA CCA GGG AAA GCC CCT AAG CTC CTG ATC
 L   A   W   Y   Q   Q   K   P   G   K   A   P   K   L   L   I>

  150         160         170         180         190
TAT AAG GCA TCT ACT TTA GAA AGT GGG GTC CCA TCA AGG TTC AGT GGC
 Y   K   A   S   T   L   E   S   G   V   P   S   R   F   S   G>

  200         210         220         230         240
AGT GGA TCT GGG ACA GAA TTC ACT CTC ACA ATC AGC AGT CTG CAA CCT
 S   G   S   G   T   E   F   T   L   T   I   S   S   L   Q   P>

  250         260         270         280
GAA GAT TTT GCA ACT TAC TAC TGT CAA CAG AGT TAC AGT ACC CCT CGA
 E   D   F   A   T   Y   Y   C   Q   Q   S   Y   S   T   P   R>

  290         300         310         320
ACG TTC GGC CAA GGG ACC AAA GTG GAT ATC AAA CGT
 T   F   G   Q   G   T   K   V   D   I   K   R
```

Fig.20.

Fig.21.
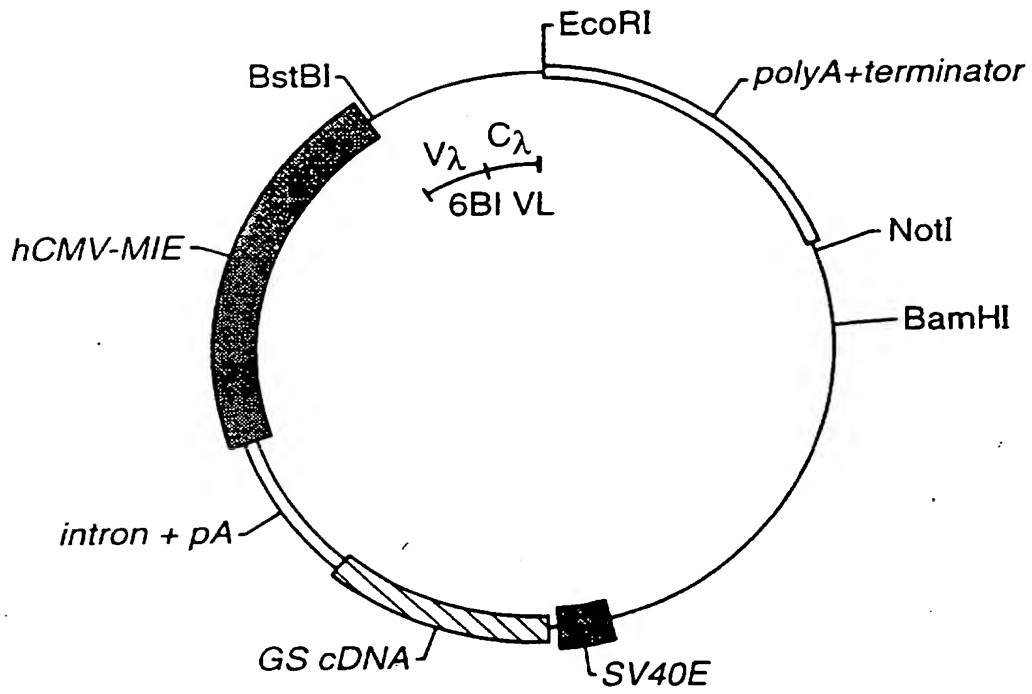
Fig.22.
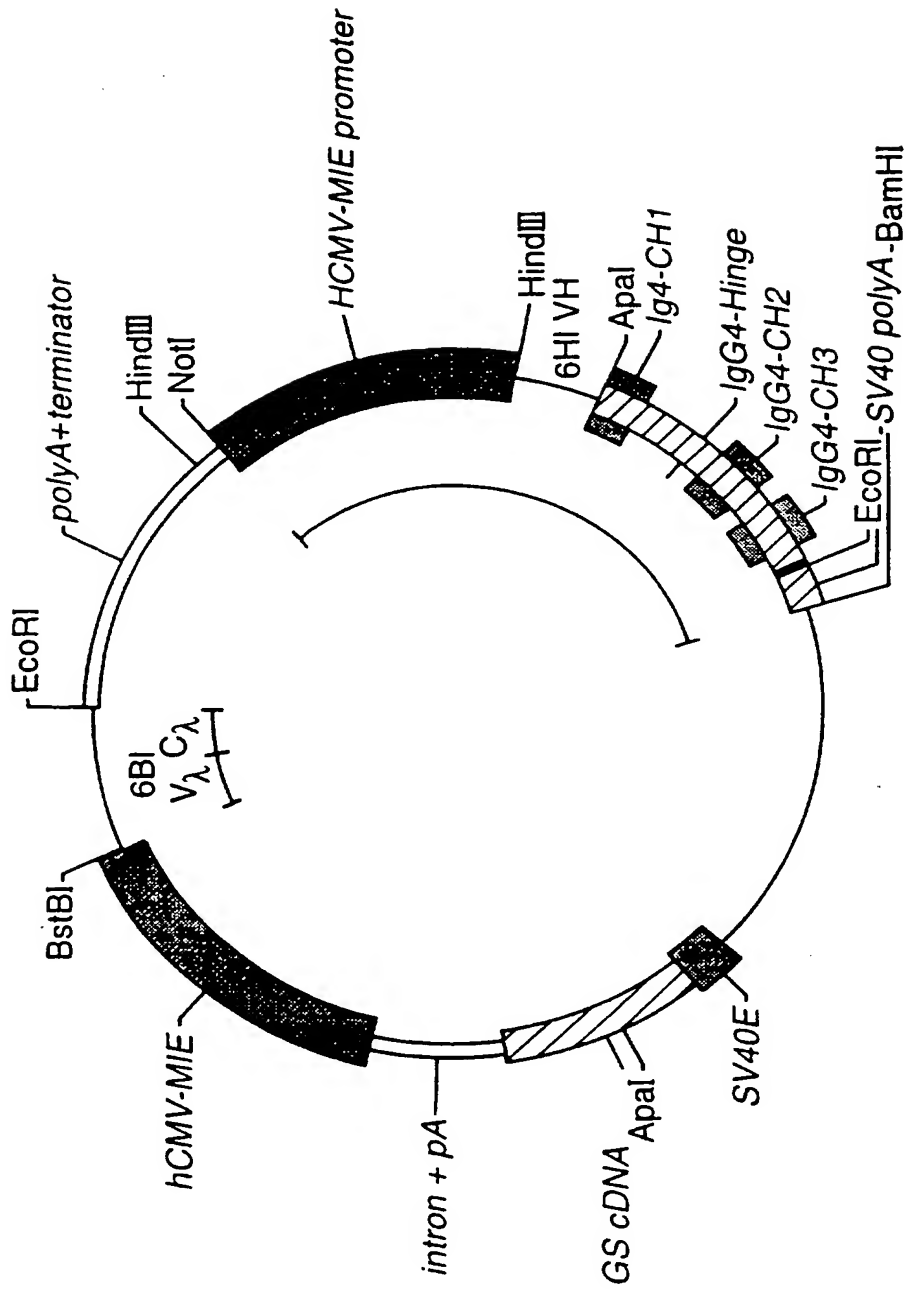
Fig.23.
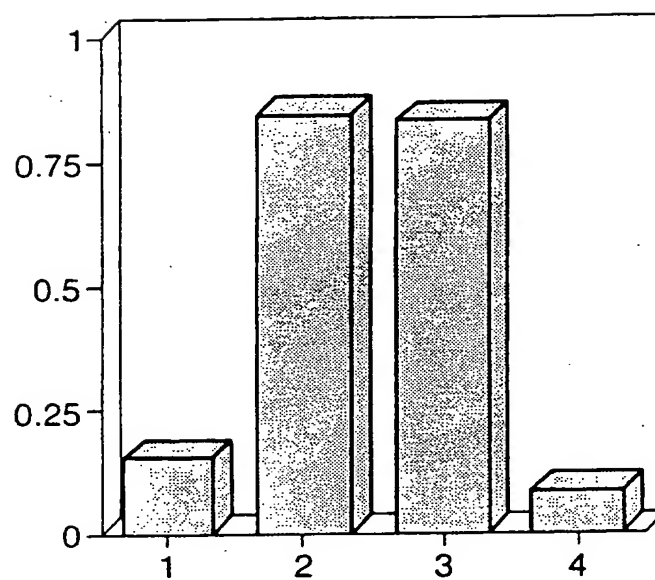
SPECIFIC BINDING MEMBERS FOR HUMAN TRANSFORMING
GROWTH FACTOR BETA; MATERIALS AND METHODS

This invention relates to specific binding

members for human transforming growth factor beta

5   (TGFβ) and materials and methods relating thereto. In

particular, it relates to specific binding members

comprising antibody binding domains; for example,

human antibodies. Human antibodies against human TGFβ

may be isolated and utilised in the treatment of

10   disease, particularly fibrotic disease and also

immune/inflammatory diseases. The isolation of

antiself antibodies from antibody segment repertoires

displayed on phage has been described (A.D.Griffiths

et al. EMBO J. 12, 725-734, 1993; A. Nissim et al.

15   EMBO J. 13, 692-698, 1994; A.D. Griffiths et al. 13,

3245-3260, 1994; C.Barbas et al. Proc. Natl. Acad.

Sci. USA 90, 10003-10007 1993; WO93/11236). However,

the present invention provides specific antibodies

against a particular isoforms of TGFβ, which

20   antibodies have unexpected and advantageous

properties.

TGFβ is a cytokine known to be involved in many

cellular processes such as cell proliferation and

differentiation, embryonic development, extracellular

25   matrix formation, bone development, wound healing,

hematopoiesis and immune and inflammatory

responses(A.B. Roberts & M. Sporn 1990 pp419-472 in

Handbook of Experimental Pharmacology eds M.B. Sporn &

A.B. Roberts, Springer Heidelberg; J.Massague et al.Annual Rev. Cell Biol. 6, 597-646, 1990).

The accumulation of excessive extra-cellular matrix is associated with various fibrotic diseases.

5   Thus there is a need to control agents such as TGFβ1 and TGFβ2 to prevent their deleterious effects in such diseases and this is one application of human antibodies to human TGFβ.

The modulation of immune and inflammatory responses by TGFbetas includes (i) inhibition of

10   proliferation of all T-cell subsets (ii) inhibitory effects on proliferation and function of B lymphocytes (iii) down-regulation of natural-killer cell activity and the T-cell response (iv) regulation of cytokine

15   production by immune cells (v) regulation of macrophage function and (vi) leucocyte recruitment and activation.

A further application of antibodies to TGFβ may be in the treatment of immune/inflammatory diseases

20   such as rheumatoid arthritis, where these functions need to be controlled.

It is a demanding task to isolate an antibody fragment specific for TGFβ of the same species. Animals do not normally produce antibodies to self

25   antigens, a phenomenon called tolerance (G.J. Nossal Science 245, 147-153, 1989). In general, vaccination with a self antigen does not result in production of circulating antibodies. It is therefore difficult to

raise human antibodies to human self antigens. There

are also in addition, ethical problems in vaccinating

humans. In relation to the raising of non-human

antibodies specific for TGFβ, there are a number of

5   problems. TGFβ is an immunosuppressive molecule and

further, there is strong conservation of sequence

between human and mouse TGFβ molecules. Mouse and

human TGFβ1 only differ by one amino acid residue, an

alanine (human) to serine (mouse) change at a buried

10   residue (R.Derynck et al. J.Biol. Chem. 261, 4377-

4379, 1986). Mouse and human TGFβ2 only differ at

three residues; residue 59 (T mouse, S human); residue

60 (K mouse, R human) and residue 94 (N mouse; K

human). This makes it difficult to raise antibodies

15   in mice against human TGFβ. Further, any antibodies

raised may only be directed against a restricted set

of epitopes.

      Polyclonal antibodies binding to human TGFβ1 and

human TGFβ2 against both neutralising and non-

20   neutralising epitopes have been raised in rabbit

(Danielpour et al. Growth Factors 2 61-71, 1989; A.

Roberts et al. Growth Factors 3, 277-286, 1990),

chicken (R&D Systems, Minneapolis) and turkey

(Danielpour et al. J. Cell Physiol. 138, 79-86, 1989).

25   Peptides representing partial TGFβ sequences have also

been used as immunogens to raise neutralising

polyclonal antisera in rabbits (W.A Border et al.

Nature 346, 371-374, 1990; K.C. Flanders Biochemistry

27, 739-746, 1988; K.C. Flanders *et al*, Growth Factors
3, 45-52, 1990). In addition there have been limited
reports of isolation of mouse monoclonals against
TGFβ. Following immunisation with bovine TGFβ2

5   (identical to human TGFβ2), three non-neutralising
monoclonal antibodies were isolated that are specific
for TGFβ2 and one neutralising antibody that is
specific for TGFβ1 and TGFβ2 (J.R. Dasch et al. J.
Immunol. 142, 1536-1541, 1989). In another report,

10   following immunisation with human TGFβ1, neutralising
antibodies were isolated which were either specific
for TGFβ1 or cross-reeacted with TGFβ1, TGFβ2 and
TGFβ3 (C. Lucas et al. J.Immunol. 145, 1415-1422,
1990). A neutralising mouse monoclonal antibody which

15   binds both TGFβ2 and TGFβ3 isoforms is available
commercially from Genzyme Diagnostics.

The present text discloses the first isolation of
human antibodies directed against human TGFβ1 and
against human TGFβ2. A mouse monoclonal antibody

20   directed against human TGFβ1 is available from R&D
Systems. This antibody only weakly neutralises TGFβ1
in a neutralisation assay. Neutralising mouse
monoclonal antibodies have also been generated from
mice immunised with human TGFβ1 peptides comprising

25   amino acid positions 48 to 60 (antibody reactive with
TGFβ1, TGfβ2 and TGFβ3)and amino acid positions 86-101
(antibody specific for TGFβ1; M. Hoefer & F.A. Anderer
Cancer Immunol. Immunother. 41, 302-308, 1995).

Phage antibody technology (WO92/01047;

PCT/GB92/00883; PCT/GB92/01755; WO93/11236) offers the

ability to isolate directly human antibodies against

human TGFβ. In application WO93/11236 the isolation

5   of antiself antibodies from phage display libraries

was disclosed and it was suggested that antibodies

specific for TGFβ could be isolated from phage display

libraries.

The present application shows that antibodies of

10   differing specificities for TGFβ molecules may be

isolated. TGFβ1, TGFβ2 and TGFβ3 are a closely

related group of cytokines. They are dimers

consisting of two 112 amino acid monomers joined by an

interchain disulphide bridge. TGFβ1 differs from

15   TGFβ2 by 27 mainly conservative changes and from TGFβ3

by 22 mainly conservative changes. These differences

have been related to the 3D structure (M.Schlunegger &

M.G.Grutter Nature 358, 430-434, 1992). The present

applicants have isolated antibodies which are

20   essentially specific for TGFβ1 (very low cross-

reactivity with TGFβ2); antibodies which are

essentially specific for TGFβ2 (very low cross-

reactivity TGFβ1); and antibodies which bind both

TGFβ1 and TGFβ2. Hence, these three different types

25   of antibodies, each type with distinctive binding

specificities must recognise different epitopes on the

TGFβ molecules. These antibodies have low cross-

reactivity with TGFβ3 as assessed by binding studies

using biosensor assays (e.g.BIACore™), ELISA and

radioreceptor assays. The most extensively studied

antibody, 6B1 IgG4, shows 9% cross-reactivity with

TGFβ3 as compared with TGFβ2, as determined by their

5 relative dissociation constants, determined using a

biosensor.

TGFβ isoforms are initially exported from cells

as inactive, latent forms (R. Pircher et al, Biochem.

Biophys. Res. Commun. 136, 30-37, 1986; L.M. Wakefield

10 et al., Growth Factors 1, 203-218, 1989). These

inactive forms are activated by proteases in plasma to

generate the active form of TGFβ. It is this active

form of TGFβ2 which binds to receptors promoting the

deposition of extracellular matrix and the other

15 biological effects of TGFβ. The active form of TGFβ

represents a relatively low proportion of TGFβ that is

in the plasma. Therefore, for a neutralising antibody

against TGFβ to be most effective at preventing

fibrosis the antibody should recognise the active but

20 not the latent form. In Example 6, it is demonstrated

that a preferred antibody of this invention ("6B1

IgG4") recognises the active but not the latent form

of TGFβ2.

The epitope of 6B1 IgG4 has been identified using

25 a combination of peptide display libraries and

inhibition studies using peptides from the region of

TGFβ2 identified from phage selected from the peptide

phage display library. This is described in Examples

11 and 14. The sequence identified from the peptide library is RVLSL and represents amino acids 60 to 64 of TGFβ2 (Example 11). The antibody 6B1 IgG4 has also been shown to bind to a peptide corresponding to amino

5 acids 56 to 69 of TGFβ2 (TQHSRVLSLYNTIN) with a three amino acid (CGG) extension at the N-terminus. RVLSL is the minimum epitope, 6B1 IgG4 is likely to bind to further adjacent amino acids. Indeed, if the epitope is three dimensional there may be other non-contiguous

10 sequences to which the antibody will bind. 6B1 IgG4 shows much weaker binding to the peptide corresponding to amino acids 56 to 69 of TGFβ1 (CGG-TQYSKVLSLYNQHN).

The results of Example 14 support the assignment of the epitope of 6B1 IgG4 on TGFβ2 to the aminoacids

15 in the region of residues 60 to 64. The peptide used in this example, residues 56 to 69, corresponds to the amino acids of alpha helix H3 (M.P. Schlunegger & M.G. Grutter Nature 358 430-434, 1992; also known as the α3 helix (S. Daopin et al Proteins: Structure, Function

20 and Genetics 17 176-192, 1993). TGFβ2 forms a head-to-tail dimer with the alpha helix H3 (also referred to as the heel) of one subunit forming an interface with finger regions (including residues 24 to 37 and residues in the region of amino acids 91 to 95; also

25 referred to as fingers 1 and 2) from the other subunit (S. Daopin et al supra). It has been proposed that the primary structural features which interact with the TGFβ2 receptor consist of amino acids at the C-

terminal end of the alpha helix H3 from one chain
together with residues of fingers 1 and 2 of the other
chain (D.L. Griffith et al Proc. Natl. Acad. Sci. USA
93 878-883,, 1996). The identification of an epitope

5 for 6B1 IgG4 within the alpha helix H3 of TGFβ2 is
consistent with 6B1 IgG4 preventing receptor binding
and neutralising the biological activity of TGFβ2.

As noted above if the epitope for 6B1 IgG4 is
three dimensional there may be other non-contiguous

10 amino acids to which the antibody may bind.

There is earlier advice that antibodies directed
against this region of TGFβ2 may be specific for TGFβ2
and neutralise its activity. Flanders et al
(Development 113 183-191, 1991) showed that polyclonal

15 antisera could be raised in rabbits against residues
50 to 75 of mature TGFβ2 and that these antibodies
recognised TGFβ2 but the TGFβ1 in Western blots. In
an earlier paper, K.C. Flanders et al (Biochemistry 27
739-746, 1988) showed that polyclonal antisera raised

20 in rabbits against amino acids 50 to 75 of TGFβ1 could
neutralise the biological activity of TGFβ1. The
antibody isolated in this application 6B1 IgG4 is a
human antibody directed against the amino acids in
this region which neutralises the biological activity

25 of human TGFβ2. It is surprising that such a
neutralising antibody against TGFβ2 can be isolated in
humans (where immunisation with a peptide cannot be
used for ethical reasons) directly from a phage

display antibody repertoire.

The knowledge that the residues of the alpha
helix H3 form a neutralising epitope for TGFβ2 means
that phage displaying neutralising antibodies are

5   obtainable by selection from phage antibody
repertoires by binding to a peptide from this region
coupled to a carrier protein such as bovine serum
albumin or keyhole limpet haemocyanin. This approach
may be applied to select antibodies which are capable

10   of neutralising the biological activity of TGFβ1 by
selecting on the peptide TQYSKVLSLYNQHN coupled to a
carrier protein. It is possible that such an approach
may be extended to peptides from receptor binding
regions of TGFβ isoforms, other than the H3 alpha

15   helix.

It has further been demonstrated by the present
inventors that antibodies specific for TGFβ are
obtainable by isolation from libraries derived from
different sources of immunoglobulin genes: from

20   repertoires of natural immunoglobulin variable
domains, e.g. from immunised or non-immunised hosts;
and synthetic repertoires derived from germline V
genes combined with synthetic CDR3s. The properties
of these antibodies in single chain Fv and whole IgG4

25   format are described.-

As noted above WO93/11236 suggested that human
antibodies directed against human TGFβ could be
isolated from phage display libraries. Herein it is

shown that the phage display libraries from which antiself antibodies were isolated in WO93/11236 may be utilised as a source of human antibodies specific for particular human TGFβ isoforms. For instance, in

5   example 1 of the present application, the antibody 1A-E5 specific for TGFβ1 and the antibodies 2A-H11 and 2A-A9 specific for TGFβ2 were isolated from the "synthetic library" described in examples 5 to 7 of WO93/11236 and in Nissim et al. (1994; supra). Also,

10   the phage display library derived from peripheral blood lymphocytes (PBLs) of an unimmunised human (examples 1 to 3 of WO93/11236) was the source for the antibody 1B2 specific for TGFβ1. Phage display libraries made subsequently utilising antibody genes

15   derived from human tonsils and bone marrow, have also provided sources of antibodies specific for human TGFβ. Thus human TGFβ is an example of a human self antigen to which antibodies may be isolated from "large universal libraries". Human antibodies against

20   human TGFβ with improved properties can be obtained by chain shuffling for instance combining the VH domains of antibodies derived from one library with the VL domains of another library thus expanding the pool of VL partners tested for each VH domain. For instance,

25   the antibodies 6B1, 6A5 and 6H1 specific for TGFβ2 utilise the 2A-H11 VH domain isolated from the "synthetic library" combined with a light chain from the PBL library.

Thus the VH and VL domains of antibodies specific
for TGFβ can be contributed from phage display
libraries derived from rearranged V genes such as
those in PBLs, tonsil and bone marrow and from V

5   domains derived from cloned germline V segments
combined with synthetic CDRs.   There are also shown to
be a diverse range of antibodies which are specific
for TGFβ1 or TGFβ2.   The antibodies which have been
isolated both against TGFβ1 and TGFβ2 have mainly

10   utilised V genes derived from VH germlines of the VH3
family.   A wider variety of light chain variable
regions have been used, of both the lambda and kappa
types.

Individual antibodies which have been isolated

15   have unexpectedly advantageous properties.   For
example, the antibodies directed against TGFβ2   (6H1,
6A5 and 6B1) have been shown to bind to TGFβ2 with
slow off-rates (off-rate constants k_{off} of the order of
10^{-3} s^{-1} and dissociation constants of less than 10^{-8}M)

20   to neutralise TGFβ2 activity in in vitro assays and to
be potent in in vivo applications.   The antibody 6B1
IgG4 has been shown to bind specifically to TGFβ2 in
immunohistochemistry in mammalian tissues and not to
cross-react with other antigens in human tissues.   The

25   properties of these antibodies may make them
particularly suitable for therapeutic applications.
The fact that these antibodies share the same heavy
chain, shows that VH domains can be effective with a

number of different light chains, although there may
be differences in potency or subtle changes of epitope
with different light chains. As shown in Examples 3
and 4 and Tables 4 and 5, 6B1 IgG4 is the most potent

5   antibody in neutralising TGFβ2 activity in the
radioreceptor assay and the TF1 proliferation assay.
Its properties may however be expected to be
qualitatively similar to the antibodies 6A5 and 6H1
with which it shares a common VH domain. Thus the

10   reduction in neural scarring observed on treatment
with 6A5 single chain Fv and 6H1 IgG4 shown in Example
5 would be expected to be reproduced with 6B1. The
antibodies directed against TGFβ1 (particularly 1B2
and its derivatives) also have unexpectedly

15   advantageous properties. Antibody 27C1/10A6 derived
from 1B2 by chain shuffling, spiking and conversion
into whole antibody IgG4 , has been shown to be potent
in an *in vitro* scarring model. The VH domain of this
antibody was derived by site directed "spiking"

20   mutagenesis from the parent antibody 7A3. A large
number of spiked clones were obtained which show
similar properties in in vitro assays. There can be a
number of changes in CDR3 of the VH compared to 27C1,
for instance, 28A-H11 differs in 7 of the 14

25   positions, 2 of which are non-conservative changes.
Thus there may be up to 50% of the residues in the VH
CDR3 changed without affecting binding properties.

    Antibodies specific for human TGFβ1 and human

TGFβ2 have been shown to be effective in animal models for the treatment of fibrotic diseases and other diseases such as rheumatoid arthritis where TGFβ is overexpressed. Antibodies against TGFβ have been

5 shown to be effective in the treatment of glomerulonephritis (W.A Border et al. Nature 346, 371-374, 1990); neural scarring (A. Logan et al. Eur. J. Neurosci. 6, 355-363, 1994); dermal scarring (M. Shah et al. Lancet 339, 213-214 1992; M.Shah et al. J.Cell

10 Science 107, 1137-1157, 1994; M. Shah et al. 108, 985-1002, 1995); lung fibrosis (S.N. Giri et al. Thorax 48, 959-966, 1993); arterial injury (Y.G. Wolf, L.M. Rasmussen & E. Ruoslahti J. Clin. Invest. 93, 1172-1178, 1994) and rheumatoid arthritis (Wahl et al J.

15 Exp. Medicine 177, 225-230, 1993). It has been suggested that TGFβ3 acts antagonistically to TGFβ1 and TGFβ2 in dermal scarring (M.Shah et al. 1995 supra.). Therefore, antibodies to TGFβ1 or TGFβ2 with apparent low cross-reactivity to TGFβ3, as assessed by

20 binding studies using a biosensor assay (e.g BIACore™), ELISA or a radioreceptor assay, as disclosed in this application, that is to say antibodies which bind preferentially to TGFβ1 or TGFβ2 compared with TGFβ3, should be advantageous in this

25 and other conditions such as fibrotic conditions in which it is desirable to counteract the fibrosis promoting effects of TGFβ1 and TGFβ2. An antibody which cross-reacts strongly with TGFβ3 has however had

an effect in an animal model of rheumatoid arthritis (Wahl *et al.*, 1993, *supra*).

There are likely to be applications further to the above mentioned conditions, as there are several

5   other *in vitro* models of disease where antibodies against TGFβ have shown promise of therapeutic efficacy. Of particular importance may be the use of antibodies against TGFβ for the treatment of eye diseases involving ocular fibrosis, including

10   proliferative retinopathy (R.A. Pena *et al.* (ref. below) , retinal detachment and post glaucoma (P.T. Khaw *et al.*, *Eye* 8 188-195, 1994) drainage surgery. Connor *et al.* (*J. Clin. Invest* 83 1661-1666, 1989) showed that much higher levels of TGFβ2 were present

15   in vitreous aspirates from patients with intraocular fibrosis associated with proliferative retinopathy compared with patients with uncomplicated retinal detachment without ocular firbrosis and that the biological activity of this TGFβ2 could be neutralised

20   with antibodies directed against TGFβ2. Moreover, Pena *et al.* (*Invest. Ophthalmology. Vis. Sci.* 35: 2804-2808, 1994) showed that antibodies against TGFβ2 inhibit collagen contraction stimulated by TGFβ2. Contraction of the vitreous gel by fibroblasts and

25   other cell types plays a critical role in the proliferative retinopathy disease process, a process thought to be mediated by TGFβ2.

There is other evidence pointing to TGFβ2 being

the most important TGFβ isoform promoting intraocular fibrosis. TGFβ2 has been shown to be the predominant isoform of TGFβ in the neural retina, retinal pigment epithelium-choroid and vitreous of the human eye

5 (Pfeffer *et al. Exp. Eye Res.* 59: 323-333, 1994) and found in human aqueous humour in specimens from eyes undergoing cataract extraction with intraocular lens implantation (Jampel *et al. Current Eye Research* 9: 963-969, 1990). Non-transformed human retinal pigment

10 epithelial cells predominantly secrete TGFβ2 (Kvanta *Opthalmic Res.* 26: 361-367, 1994).

Other diseases which have potential for treatment with antibodies against TGFβ include adult respiratory distress syndrome, cirrhosis of the liver, post

15 myocardial infarction, post angioplasty restenosis, keloid scars and scleroderma. The increase level of expression of TGFβ2 in osteoporosis (Erlenbacher *et al. J. Cell Biol.* 132: 195-210, 1996) means that htis is a disease potentially treatable by antibodies

20 directed against TGFβ2.

The use of antibodies against TGFβ for the treatment of diseases has been the subject of patent applications for fibrotic disease (WO91/04748); dermal scarring (WO92/17206); macrophage deficiency diseases

25 (PCT/US93/00998); macrophage pathogen infections (PCT/US93/02017); neural scarring (PCT/US93/03068); vascular disorders (PCT/US93/03795); prevention of cataract (WO95/13827). The human antibodies against

human TGFβ disclosed in this application should be valuable in these conditions.

It is shown herein that the human antibodies both against human TGFβ1 and against human TGFβ2 can be

5 effective in the treatment of fibrosis in animal models of neural scarring and glomerulonephritis in either single chain Fv and whole antibody format. This is the first disclosure of the effectiveness of antibodies directed only against TGFβ2 as sole

10 treatment in these indications, although some effectiveness of antibodies against TGFβ2 only has been observed in a lung fibrosis model (Giri et al. Thorax 48, 959-966, 1993 supra). The effectiveness of the human antibodies against human TGFβ in treatment

15 of fibrotic disease has been determined by measuring a decrease in the accumulation of components of the extracellular matrix, including fibronectin and laminin in animal models.

The evidence of efficacy of the antibodies

20 against TGFβ2 and TGFβ1 describe herein in prevention of neural scarring in the animal model experiment means that these antibodies are likely to be effective in other disease states mediated by TGFβ. For comparison, antisera isolated from turkeys directed

25. against TGFβ isoforms by Danielpour et al. (Cell Physiol. 138: 79-86, 1989) have been shown to be effective in the prevention of dermal scarring (Shah et al. J. Cell Science 108: 985-1002, 1995), neural

scarring (Logan *et al., supra*) and in *in vitro*
experiments relating to proliferative retinopathy
(Connor *et al., supra*).

5   *TERMINOLOGY*

Specific binding member

This describes a member of a pair of molecules
which have binding specificity for one another. The
10   members of a specific binding pair may be naturally
derived or wholly or partially synthetically produced.
One member of the pair of molecules has an area on its
surface, or a cavity, which specifically binds to and
is therefore complementary to a particular spatial and
15   polar organisation of the other member of the pair of
molecules. Thus the members of the pair have the
property of binding specifically to each other.
Examples of types of specific binding pairs are
antigen-antibody, biotin-avidin, hormone-hormone
20   receptor, receptor-ligand, enzyme-substrate. This
application is concerned with antigen-antibody type
reactions.

Antibody

25   This describes an immunoglobulin whether natural
or partly or wholly synthetically produced. The term
also covers any polypeptide or protein having a
binding domain which is, or is homologous to, an

antibody binding domain. These can be derived from natural sources, or they may be partly or wholly synthetically produced. Examples of antibodies are the immunoglobulin isotypes and their isotypic

5 subclasses; fragments which comprise an antigen binding domain such as Fab, scFv, Fv, dAb, Fd; and diabodies.

It is possible to take monoclonal and other antibodies and use techniques of recombinant DNA

10 technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an

15 antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP-A-184187, GB 2188638A or EP-A-239400. A hybridoma or other cell producing an antibody may be subject to genetic mutation or other

20 changes, which may or may not alter the binding specificity of antibodies produced.

As antibodies can be modified in a number of ways, the term "antibody" should be construed as covering any specific binding member or substance

25 having a binding domain with the required specificity. Thus, this term covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including any polypeptide comprising an

immunoglobulin binding domain, whether natural or wholly or partially synthetic. Chimeric molecules comprising an immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore

5 included. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023.

It has been shown that fragments of a whole antibody can perform the function of binding antigens.

10 Examples of binding fragments are (i) the Fab fragment consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward, E.S.

15 et al., Nature 341, 544-546 (1989)) which consists of a VH domain; (v) isolated CDR regions; (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a

20 peptide linker which allows the two domains to associate to form an antigen binding site (Bird et al, Science, 242, 423-426, 1988; Huston et al, PNAS USA, 85, 5879-5883, 1988); (viii) bispecific single chain Fv dimers (PCT/US92/09965) and (ix) "diabodies",

25 multivalent or multispecific fragments constructed by gene fusion (WO94/13804; P. Holliger et al Proc. Natl. Acad. Sci. USA 90 6444-6448, 1993).

Diabodies are multimers of polypeptides, each

polypeptide comprising a first domain comprising a binding region of an immunoglobulin light chain and a second domain comprising a binding region of an immunoglobulin heavy chain, the two domains being

5   linked (e.g. by a peptide linker) but unable to associate with each other to form an antigen binding site: antigen binding sites are formed by the association of the first domain of one polypeptide within the multimer with the second domain of another

10   polypeptide within the multimer (WO94/13804).

Where bispecific antibodies are to be used, these may be conventional bispecific antibodies, which can be manufactured in a variety of ways (Holliger, P. and Winter G. Current Opinion Biotechnol. 4, 446-449

15   (1993)), eg prepared chemically or from hybrid hybridomas, or may be any of the bispecific antibody fragments mentioned above. It may be preferable to use scFv dimers or diabodies rather than whole antibodies. Diabodies and scFv can be constructed

20   without an Fc region, using only variable domains, potentially reducing the effects of anti-idiotypic reaction. Other forms of bispecific antibodies include the single chain "Janusins" described in Traunecker et al, Embo Journal, 10, 3655-3659, (1991).

25   Bispecific diabodies, as opposed to bispecific whole antibodies, may also be particularly useful because they can be readily constructed and expressed in E.coli. Diabodies (and many other polypeptides

such as antibody fragments) of appropriate binding
specificities can be readily selected using phage
display (WO94/13804) from libraries. If one arm of
the diabody is to be kept constant, for instance, with
5   a specificity directed against antigen X, then a
library can be made where the other arm is varied and
an antibody of appropriate specificity selected.

Antigen binding domain

10   This describes the part of an antibody which
comprises the area which specifically binds to and is
complementary to part or all of an antigen. Where an
antigen is large, an antibody may only bind to a
particular part of the antigen, which part is termed
15 an epitope. An antigen binding domain may be provided
by one or more antibody variable domains. Preferably,
an antigen binding domain comprises an antibody light
chain variable region (VL) and an antibody heavy chain
variable region (VH).

20

Specific

This may be used to refer to the situation in
which one member of a specific binding pair will not
show any significant binding to molecules other than
25 its specific binding partner(s). The term is also
applicable where e.g. an antigen binding domain is
specific for a particular epitope which is carried by
a number of antigens, in which case the specific

binding member carrying the antigen binding domain will be able to bind to the various antigens carrying the epitope.

5   *Neutralisation*

This refers to the situation in which the binding of a molecule to another molecule results in the abrogation or inhibition of the biological effector function of the another molecule.

10

Functionally equivalent variant form

This refers to a molecule (the variant) which although having structural differences to another molecule (the parent) retains some significant

15   homology and also at least some of the biological function of the parent molecule, e.g. the ability to bind a particular antigen or epitope. Variants may be in the form of fragments, derivatives or mutants. A variant, derivative or mutant may be obtained by

20   modification of the parent molecule by the addition, deletion, substitution or insertion of one or more amino acids, or by the linkage of another molecule. These changes may be made at the nucleotide or protein level. For example, the encoded polypeptide may be a

25   Fab fragment which is then linked to an Fc tail from another source. Alternatively, a marker such as an enzyme, flourescein, etc, may be linked.

Comprise

This is generally used in the sense of include, that is to say permitting the presence of one or more features or components.

5

The present invention generally provides a specific binding member comprising an antibody antigen binding domain. More particularly it provides a specific binding member for TGFβ, particularly the

10   isoforms TGFβ2, TGFβ1, or TGFβ1 and TGFβ2.

The present invention provides a specific binding member which comprises a human antibody antigen binding domain specific for TGFβ1 and/or TGFβ2 and which has low cross reactivity with TGFβ3. The cross-

15   reactivity may be as assessed using any or all of the following assays: biosensor (e.g. BIACore™), ELISA and radioreceptor. The present invention provides specific binding member which comprises a human antibody antigen binding domain specific for TGFβ1

20   and/or TGFβ2 which binds preferentially to these isoforms compared with TGFβ3.

The TGFβ may be human TGFβ.

The specific binding member may be in the form of an antibody fragment such as single chain Fv (scFv).

25   Other types of antibody fragments may also be utilised such as Fab, Fab', F(ab')$_2$, Fabc, Facb or a diabody (G.Winter & C.Milstein Nature 349, 293-299, 1991; WO94/13804). The specific binding member may be in

the form of a whole antibody. The whole antibody may
be in any of the forms of the antibody isotypes eg
IgG, IgA, IgE, and IgM and any of the forms of the
isotype subclasses eg IgG1 or IgG4.

5   The specific binding member may also be in the
form of an engineered antibody eg bispecific antibody
molecules (or fragments such as F(ab')$_2$) which have
one antigen binding arm (ie specific binding domain)
against TGFβ and another arm against a different
10   specificity. Indeed the specific binding members
directed against TGFβ1 and/or TGFβ2 described herein
may be combined in a bispecific diabody format. For
example the antibodies 31G9 directed against TGFβ1 and
6H1 directed against TGFβ2 may be combined to give a
15   single dimeric molecule with both specificities.

The binding domain may comprise part or all of a
VH domain encoded by a germ line gene segment or a re-
arranged gene segment. The binding domain may
comprise part or all of either a VL kappa domain or a
20   VL lambda domain.

The binding domain may be encoded by an altered
or variant form of a germ line gene with one or more
nucleotide alterations (addition, deletion,
substitution and/or insertion), e.g. about or less
25   than about 25, 20, 15, 10 or 5 alterations, 4, 3, 2 or
1, which may be in one or more frameworks and/or
CDR's.

The binding domain may comprise a VH3 gene

sequence of one of the following germ lines; the DP49 germ line; the DP53 germ line; the DP50 germ line; the DP46 germ line; or a re-arranged form thereof.

A preferred VH domain for anti-TGFβ2 specific binding members according to the present invention is that of 6H1 VH, whose sequence is shown in Figure 2(a)(i). 6H1 may be paired with a variety of VL domains, as exemplified herein. Amino acid sequence variants of 6H1 VH may be employed.

The specific binding member may neutralise the *in vitro* and/or *in vivo* effect of TGFβ, that is one or more of the isoforms, particularly TGFβ1 and/or TGFβ2.

The specific binding member may be a high affinity antibody. Preferred affinities are discussed elsewhere herein.

The binding domain may comprise part or all of a VH domain having either an amino acid sequence as shown in Fig 1(a)(i) or (ii) or Fig 1(c)(i) or a functionally equivalent variant form of a said amino acid sequence.

The binding domain may comprise part or all of a VH domain encoded by either a nucleotide sequence as shown in Fig 1(a)(i) or (ii) or Fig 1(c)(i) or a functionally equivalent variant form of a said nucleotide sequence.

The binding domain may comprise part or all of a VL domain having either an amino acid sequence as shown in Fig 1(a)(iii) or Fig 1(b) or a functionally

equivalent variant form of a said amino acid sequence.

The binding domain may comprise part or all of a VL domain encoded by either a nucleotide sequence as shown in Fig 1(a)(iii) or Fig 1(b) or a functionally
5   equivalent variant form of a said nucleotide sequence.

The binding domain may comprise part or all of a VH domain having a variant form of the Fig 1(a)(i) amino acid, the variant form being one of those as provided by Fig 3.

10   The binding domain may comprise part or all of a VH domain having either an amino acid sequence as shown in Fig 2(a)(i) or (ii) or a functionally equivalent variant form of a said amino acid sequence.

The binding domain may comprise part or all of a
15   VH domain encoded by either a nucleotide sequence as shown in Fig 2(a)(i) or (ii) or a functionally equivalent variant form of a said nucleotide sequence.

The binding domain may comprise part or all of a VL domain having either an amino acid sequence as
20   shown in any of Figs 2(b)(i) to (v) or a functionally equivalent variant form of a said amino acid sequence.

The binding domain may comprise part or all of a VL domain encoded by either a nucleotide sequence as shown in any of Figs 2(b)(i) to (v) or a functionally
25   equivalent variant form of a said nucleotide sequence.

The binding domain may be specific for both TGFβ1 and TGFβ2. The binding domain may be specific for both human TGFβ1 and human TGFβ2. The specific

binding member may be in the form of scFv.

The binding domain may comprise part or all of a VL domain having either an amino acid sequence as shown in Fig 4 or a functionally equivalent variant

5 form of said amino acid sequence. The binding domain may comprise part or all of a VL domain encoded by either the nucleotide sequence as shown in Fig 4 or a functionally equivalent variant form of said nucleotide sequence.

10 In particular, the binding domain may comprise one or more CDR (complementarity determining region) with an amino acid sequence shown in any of the figures. In a preferred embodiment, the binding domain comprises one or more of the CDRs, CDR1, CDR2

15 and/or CDR3 shown in the Figures, especially any of those shown in Figure 19. In a preferred embodiment, the binding domain comprises a VH CDR3 sequence as shown, especially as shown in Figure 19. Functionally equivalent variant forms of the CDRs are encompassed

20 by the present invention, in particular variants which differ from the CDR sequences shown by addition, deletion, substitution or insertion of one or more amino acids and which retain ability to bind the antigen and optionally one or more of the preferred

25 characteristics for specific binding members of the present invention as disclosed herein. The specific binding member may comprise all or part of the framework regions shown flanking and between the CDRs

in the Figures, especially Figure 19, or different framework regions including modified versions of those shown.

So-called "CDR-grafting" in which one or more CDR
5   sequences of a first antibody is placed within a framework of sequences not of that antibody, e.g. of another antibody is disclosed in EP-B-0239400.

The present invention also provides a polypeptide with a binding domain specific for TGFβ which
10 polypeptide comprises a substantial part or all of either an amino acid sequence as shown in any of Fig 1(a), Fig 1(b), Fig 1(c), Fig 2(a), Fig 2(b), Fig 4 or a functionally equivalent variant form of a said amino acid sequence. The polypeptide may comprise a
15 substantial part or all of an amino acid sequence which is a functionally equivalent variant form of the Fig 1(a)(i) amino acid sequence, the variant being one of those variants as shown in Fig 3.

Variable domain amino acid sequence variants of
20 any of the VH and VL domains whose sequences are specifically disclosed herein may be employed in accordance with the present invention, as discussed. Particular variants may include one or more amino acid sequence alterations (addition, deletion, substitution
25 and/or insertion), maybe less than about 20 alterations, less than about 15 alterations, less than about 10 alterations or less than about 5 alterations, 4, 3, 2 or 1. Alterations may be made in one or more

framework regions and/or one or more CDR's.

A specific binding member according to the invention may be one which competes for binding to TGFβ1 and/or TGFβ2 with any specific binding member

5   which both binds TGFβ1 and/or TGFβ2 and comprises part of all of any of the sequences shown in the Figures. Competition between binding members may be assayed easily *in vitro*, for example by tagging a specific reporter molecule to one binding member which can be

10   detected in the presence of other untagged binding member(s), to enable identification of specific binding members which bind the same epitope or an overlapping epitope.

Preferred specific binding members for TGFβ1

15   compete for binding to TGFβ1 with the antibody CS37, discussed in more details elsewhere herein.

Preferred specific binding members for TGFβ2 compete for binding to TGFβ2 with the antibody 6B1 discussed in more detail elsewhere herein. They may

20   bind the epitope RVLSL or a peptide comprising the amino acid sequence RVLSL, particularly such a peptide which adopts an α-helical conformation. They may bind the peptide TQHSRVLSLYNTIN. In testing for this, a peptide with this sequence plus CGG at the N-terminus

25   may be used. Specific binding members according to the present invention may be such that their binding for TGFβ2 is inhibited by a peptide comprising RVLSL, such as a peptide with the sequence TQHSRVLSLYNTIN.

In testing for this, a peptide with this sequence plus CGG at the N-terminus may be used.

TQHSRVLSLYNTIN corresponds to the alpha helix H3 (residues 56-69) of TGFβ2, as discussed elsewhere herein. The equivalent region in TGFβ1 has the sequence TQYSKVLSLYNQHN. Anti-TGFβ1 antibodies which bind this region are of particular interest in the present invention, and are obtainable for example by panning a peptide with this sequence (or with CGG at the N-terminus) against a phage display library. Specific binding members which bind the peptide may be selected by means of their binding, and may be neutralising for TGFβ1 activity. Binding of such specific binding members to TGFβ1 may be inhibited by the peptide TQYSKVLSLYNQHN (optionally with CGG at the N-terminus).

A specific binding member according to the present invention which is specific for TGFβ2 may show no or substantially no binding for the latent form of TGFβ2, i.e. be specific for the active form of TGFβ2. 6B1 is shown in Example 6 to have this property.

6B1 is particularly suitable for therapeutic use in the treatment of fibrotic disorders because it has the following advantageous properties. 6B1 binds to TGFβ2 with a dissociation constant of 2.3nM in the single chain form and 0.89nM for the whole antibody form, 6B1 IgG4 (Example 13). The antibody 6B1 IgG4 neutralises the biological activity of TGFβ2 in an

antiproliferation assay (IC_{50} 2nM; examples 7 and 10)
and in a radioreceptor assay (IC_{50} less than 1nM; Table
6). The antibody binds to the peptide TQHSRVLSLYNTIN
($TGF\beta2_{56-69}$) from the alpha helix H3 of $TGF\beta2$ and

5 recognises the corresponding peptide from $TGF\beta1$ more
weakly. 6B1 recognises the active but not the latent
form of $TGF\beta2$ (Example 6), recognises $TGF\beta2$ in
mammalian tissues by ICC and does not bind non-
specifically to other human tissues (Example 12). The

10 antibody preferentially binds to $TGF\beta2$ as compared to
$TGF\beta3$, the cross-reactivity with $TGF\beta3$ being 9% as
determined by the ratio of the dissociation constants.

The other antibodies described in this
application which contain the 6H1 VH domain, 6H1 and

15 6A5 have similar properties. The dissociation
constants of were determined to be 2nM for 6B1 IgG4
(Example 2) and 0.7nM for 6A5 single chain Fv (Table
1). 6H1 IgG4 neutralises the biological activity of
$TGF\beta2$ with IC_{50} values of 12 to 15nM (Examples 7 and

20 10). 6A5 and 6H1 inhibit receptor binding of $TGF\beta2$ in
a radioreceptor assay with IC_{50} values of about 1nM in
the single chain Fv format and 10nM or below in the
whole antibody, IgG4 format. Both 6H1 IgG4 and 6A5
scFv were shown to be effective in the prevention of

25 neural scarring (Example 5).

Therefore for the first human antibodies directed
against $TGF\beta2$ are provided which have suitable
properties for treatment of diseases characterised by

the deleterious presence of TGFβ2. Such antibodies
preferably neutralise TGFβ2 and preferably have a
dissociation constant for TGFβ2 of less than about
100nM, more preferably about 10nM, more preferably

5   below about 5nM. The antibodies preferentially bind
to TGFβ2 as compared to TGFβ3, preferably have less
than 20% cross-reactivity with TGFβ3 (as measured by
the ratio of the dissociation constants) and
preferably have less than about 10% cross-reactivity.

10   The antibody preferably recognises the active but not
the latent form of TGFβ2.

For antibodies against TGFβ1, the properties
desired for an antibody to be effective in treatment
of fibrotic disease are similar. Such antibodies

15   preferably neutralise TGFβ1 and have a dissociation
constant for TGFβ1 of less than about 100nM, more
preferably below about 10nM, more preferably below
about 5nM. The antibodies preferentially bind to
TGFβ1 as compared to TGFβ3, preferably have less than

20   about 20% cross-reactivity with TGFβ3 (as measured by
the ratio of the dissociation constants) and more
preferably have less than about 10% cross-reactivity.
The antibody preferably recognises the active but not
the latent form of TGFβ1. The antibody 31G9 has a

25   dissociation constant of 12nM (Table 5). The
antibodies CS37 scFv and 27C1/10A6 IgG4 show IC_{50}
values in a radioreceptor assay of 8nM and 9nM
respetively, indicating a dissociation contstant in

the low nanomolar range. 27C1/10A6 IgG4 was shown to be effective in a neural scarring model. Cross-reactivity of antibodies of the 1B2 lineage with TGFβ3 is very low (Example 9).

5   In addition to an antibody sequence, the specific binding member may comprise other amino acids, e.g. forming a peptide or polypeptide, or to impart to the molecule another functional characteristic in addition to ability to bind antigen. For example, the specific

10   binding member may comprise a label, an enzyme or a fragment thereof and so on.

The present invention also provides a polynucleotide which codes for a polypeptide with a binding domain specific for TGFβ which polynucleotide

15   comprises a substantial part or all of a nucleotide sequence which codes for either an amino acid sequence as shown in any one of Fig 1(a), Fig 1(b), Fig 1(c), Fig 2(a), Fig 2(b), Fig 4 or a functionally equivalent variant form of a said amino acid sequence. The

20   polynucleotide may code for a polypeptide with a binding domain specific for TGFβ which polynucleotide comprises a substantial part or all of a nucleotide sequence which codes for an amino acid sequence which is a functionally equivalent variant form of the Fig

25   1(a)(i) amino acid sequence, the variant being one of those as shown in Fig 3. The polynucleotide may code for a polypeptide with a binding domain specific for TGFβ which polynucleotide comprises a substantial

part or all of a either a nucleotide sequence as shown
in any of Fig 1(a), Fig 1(b), Fig 1(c), Fig 2(a), Fig
2(b), Fig 4 or a functionally equivalent variant form
of said nucleotide sequence. The polynucleotide may

5   code for a polypeptide with a binding domain specific
for TGFβ which polynucleotide comprises a substantial
part or all a nucleotide sequence which codes for a
variant form of the Fig 1(a)(i) amino acid sequence,
the variant being one of those as shown in Fig 3.

10   The present invention also provides constructs in
the form of plasmids, vectors, transcription or
expression cassettes which comprise least one
polynucleotide as above.

The present invention also provides a recombinant
15   host cell which comprises one or more constructs as
above.

A specific binding member according to the
present invention may be made by expression from
encoding nucleic acid. Nucleic acid encoding any

20   specific binding member as provided itself forms an
aspect of the present invention, as does a method of
production of the specific binding member which method
comprises expression from encoding nucleic acid
therefor. Expression may conveniently be achieved by

25   culturing under appropriate conditions recombinant
host cells containing the nucleic acid. Following
production by expression a specific binding member may
be isolated and/or purified using any suitable

technique, then used as appropriate.

Specific binding members and encoding nucleic acid molecules and vectors according to the present invention may be provided isolated and/or purified,

5 e.g. from their natural environment, in substantially pure or homogeneous form, or, in the case of nucleic acid, free or substantially free of nucleic acid or genes origin other than the sequence encoding a polypeptide with the required function. Nucleic acid

10 according to the present invention may comprise DNA or RNA and may be wholly or partially synthetic. The term "isolate" encompasses all these possibilities.

The nucleic acid may encode any of the amino acid sequences shown in any of the Figures, or any

15 functionally equivalent form. The nucleotide sequences employed may be any of those shown in any of the Figures, or may be a variant, allele or derivative thereof. Changes may be made at the nucleotide level by addition, substitution, deletion or insertion of

20 one or more nucleotides, which changes may or may not be reflected at the amino acid level, dependent on the degeneracy of the genetic code.

Systems for cloning and expression of a polypeptide in a variety of different host cells are

25 well known. Suitable host cells include bacteria, mammalian cells, yeast and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include

Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells and many others. A common, preferred bacterial host is *E. coli*.

The expression of antibodies and antibody
5   fragments in prokaryotic cells such as *E. coli* is well established in the art. For a review, see for example Plückthun, A. Bio/Technology 9: 545-551 (1991). Expression in eukaryotic cells in culture is also available to those skilled in the art as an option for
10   production of a specific binding member, see for recent reviews, for example Reff, M.E. (1993) Curr. Opinion Biotech. 4: 573-576; Trill J.J. et al. (1995) Curr. Opinion Biotech 6: 553-560.

Suitable vectors can be chosen or constructed,
15   containing appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences; marker genes and other sequences as appropriate. Vectors may be plasmids, viral e.g. 'phage, or phagemid, as
20   appropriate. For further details see, for example; *Molecular Cloning: a Laboratory Manual*: 2nd edition, Sambrook et al., 1989, Cold Spring Harbor Laboratory Press. Many known techniques and protocols for manipulation of nucleic acid, for example in
25   preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Short Protocols in Molecular Biology*, Second

Edition, Ausubel et al. eds., John Wiley & Sons, 1992.
The disclosures of Sambrook et al. and Ausubel et al.
are incorporated herein by reference.

Thus, a further aspect of the present invention

5   provides a host cell containing nucleic acid as
disclosed herein. A still further aspect provides a
method comprising introducing such nucleic acid into a
host cell. The introduction may employ any available
technique. For eukaryotic cells, suitable techniques

10 may include calcium phosphate transfection, DEAE-
Dextran, electroporation, liposome-mediated
transfection and transduction using retrovirus or
other virus, e.g. vaccinia or, for insect cells,
baculovirus. For bacterial cells, suitable techniques

15 may include calcium chloride transformation,
electroporation and transfection using bacteriophage.

The introduction may be followed by causing or
allowing expression from the nucleic acid, e.g. by
culturing host cells under conditions for expression

20 of the gene.

In one embodiment, the nucleic acid of the
invention is integrated into the genome (e.g.
chromosome) of the host cell. Integration may be
promoted by inclusion of sequences which promote

25 recombination with the genome, in accordance with
standard techniques.

The present invention also provides a method
which comprises using a construct as stated above in

an expression system in order to express a specific binding member or polypeptide as above.

Following production of a specific binding member it may be used for example in any of the manners

5 disclosed herein, such as in the formulation of a composition, pharmaceutical or a diagnostic product, such as a kit comprising in addition to the specific binding member one or more reagents for determining binding of the member to cells, as discussed. A

10 composition may comprise at least one component in addition to the specific binding member.

The present invention also provides pharmaceuticals which comprise a specific binding member as above, optionally with one or more

15 excipients.

The present invention also provides the use of a specific binding member as above in the preparation of a medicament to treat a condition in which it is advantageous to counteract the fibrosis promoting

20 effects of TGFβ. The condition may be a fibrotic condition characterized by an accumulation in a tissue of components of the extracellular matrix. The components of the extracellular matrix may be fibronectin or laminin.

25 The condition may be selected from the group consisting of: glomerulonephritis, neural scarring, dermal scarring, ocular scarring, lung fibrosis, arterial injury, proliferative retinopathy, retinal

detachment, adult respiratory distress syndrome, liver cirrhosis, post myocardial infarction, post angioplasty restenosis, keloid scarring, scleroderma, vascular disorders, cataract, glaucoma, proliferative

5   retinopathy.

The condition may be neural scarring or glomerulonephritis.

The present invention also provides the use of a specific binding member as above, in the preparation

10   of a medicament to treat an immune/inflammatory disease condition in which it is advantageous to counteract the effects of TGFβ. Illustrative conditions are rheumatoid arthritis, macrophage deficiency disease and macrophage pathogen infection.

15   The present invention also provides a method which comprises administering to a patient a therapeutically effective amount of a specific binding member as above in order to treat a condition in which it is advantageous to counteract the fibrosis

20   promoting effects of TGFβ. Fibrotic conditions are listed above.

The present invention also provides a method which comprises administering to a patient a prophylactically effective amount of a specific

25   binding member as above in order to prevent a condition in which it is advantageous to prevent the fibrosis promoting effects of TGFβ. Fibrotic conditions are listed above.

The present invention also provides methods which comprise administering to patients prophylactically and/or therapeutically effective amounts of a specific binding member as above in order to prevent or treat

5   an immune/inflammatory disease condition in which it is advantageous to counteract the effects of TGFβ. Illustrative conditions are stated above.

Thus, various aspects of the invention provide methods of treatment comprising administration of a

10 specific binding member as provided, pharmaceutical compositions comprising such a specific binding member, and use of such a specific binding member in the manufacture of a medicament for administration, for example in a method of making a medicament or

15 pharmaceutical composition comprising formulating the specific binding member with a pharmaceutically acceptable excipient.

In accordance with the present invention, compositions provided may be administered to

20 individuals, which may be any mammal, particularly rodent, e.g. mouse, horse, pig, sheep, goat, cattle, dog, cat or human. Administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit

25 may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription

of treatment, eg decisions on dosage etc, is within
the responsibility of general practioners and other
medical doctors. Appropriate doses of antibody are
well known in the art; see Ledermann J.A. et al.

5   (1991) Int J. Cancer 47: 659-664; Bagshawe K.D. et al.
(1991) Antibody, Immunoconjugates and
Radiopharmaceuticals 4: 915-922.

A composition may be administered alone or in
combination with other treatments, either

10   simultaneously or sequentially dependent upon the
condition to be treated.

Pharmaceutical compositions according to the
present invention, and for use in accordance with the
present invention, may comprise, in addition to active

15   ingredient, a pharmaceutically acceptable excipient,
carrier, buffer, stabiliser or other materials well
known to those skilled in the art. Such materials
should be non-toxic and should not interfere with the
efficacy of the active ingredient. The precise nature

20   of the carrier or other material will depend on the
route of administration, which may be oral, or by
injection, e.g. intravenous.

Pharmaceutical compositions for oral
administration may be in tablet, capsule, powder or

25   liquid form. A tablet may comprise a solid carrier
such as gelatin or an adjuvant. Liquid pharmaceutical
compositions generally comprise a liquid carrier such
as water, petroleum, animal or vegetable oils, mineral

oil or synthetic oil. Physiological saline solution,
dextrose or other saccharide solution or glycols such
as ethylene glycol, propylene glycol or polyethylene
glycol may be included.

5   For intravenous, injection, or injection at the
site of affliction, the active ingredient will be in
the form of a parenterally acceptable aqueous solution
which is pyrogen-free and has suitable pH, isotonicity
and stability. Those of relevant skill in the art are

10   well able to prepare suitable solutions using, for
example, isotonic vehicles such as Sodium Chloride
Injection, Ringer's Injection, Lactated Ringer's
Injection. Preservatives, stabilisers, buffers,
antioxidants and/or other additives may be included,

15   as required.

       Further aspects of the invention and embodiments
will be apparent to those skilled in the art. In
order that the present invention is fully understood,

20   the following examples are provided by way of
exemplification only and not by way of limitation.
       Reference is made to the following figures.

       Figure 1 shows the DNA and protein sequences of

25   antibodies specific for TGFβ1. Figure 1(a) shows the
amino acid and encoding nucleic acid sequences of
antibody variable domains of antibodes to TGFβ1
isolated directly from repertoires: Figure 1(a)(i)-

1B2 VH (also known as 7A3 VH); Figure 1(a) (ii) - 31G9 VH; Figure 1(a) (iii) - 31G9 VL. Figure 1 (b) shows the amino acid and encoding nucleic acid sequences of antibody light chain variable domains of antibodies to

5   TGFβ1 isolated by chain shuffling: Figure 1(b) (i) - 7A3 VL; Figure 1(b) (ii) - 10A6 VL. Figure 1(c) (i) shows the amino acid and encoding nucleic acid sequences for 27C1 VH, from an antibody to TGFβ1 isolated from a CDR3 spiking experiment.

10   Figure 2 shows the DNA and protein sequences of antibodies specific for TGFβ2. Figure 2(a) shows amino acid and encoding nucleic acid sequences for variable domains of antibodies to TGFβ2 isolated directly from repertoires: Figure 2(a) (i) - 2A-H11 VH

15   (also known as 6H1 VH); Figure 2(a) (ii) - 2A-A9 VH (also known as 11E6 VH). Figure 2(b) shows amino acid and encoding nucleic acid sequences of antibody variable domains of antibodies specific for TGFβ2 isolated following chain shuffling: Figure 2(b) (i) -

20   6H1 VL; Figure 2(b) (ii) - 6A5 VL; Figure 2(b) (iii) - 6B1 VL; Figure 2(b) (iv) 11E6 VL; (v) Figure 2(b) (v) - 14F12 VL.

Figure 3 shows the protein sequences of VH CDR3 of clones derived from 1B2 by 'spiking' mutagenesis.

25   Differences from 1B2 VH CDR3 are in bold.

Figure 4 shows the DNA and protein sequence of the VH and VL domains of VT37, cross-reactive between TGFβ1 and TGFβ2.

Figure 5 shows the DNA sequence and encoded amino acid sequence in the region of the heavy chain VH leader from the vector vhcassette2. Restriction enzymes HindIII, SfiI, PstI, BstEII, BamHI and EcoRI

5   cut at the points indicated.

Figure 6 shows a map of the vector pG4D100 (not to scale). Multiple cloning site (MCS): 5'-HindIII-PacI-BamHI-(XanI)-(PmlI)-(NheI)-AscI-(BssHII)-XhoI-PmeI-BsiWI-3'. Restriction sites shown in brackets

10   are not unique.

Figure 7 shows the DNA sequence, including intron, and encoded amino acid sequence in the region of the light chain VL leader for the vector vlcassette1 (vlcassette CAT1). Restrcition enzymes

15   HindIII, ApaLI, SacI, XhoI and BamHI cut at the sites indicated (ApaLI within the leader).

Figure 8 shows a map of the vector pLN10 (not to scale). Multiple cloning site (MCS): 5'-HindIII-(SphI)-(PstI)-SalI-XbaI-BamHI-3' (1224-1259.

20   Restriction sites shown in brackets are not unique.

Figure 9 shows a map of the vector pKN100 (not to scale). Multiple cloning site (MCS): 5'-MluI-(AvaI)-HindIII-(SphI)-(PstI)-SalI-XbaI-BamHI-3'. Restriction sites shown in brackets are not unique.

25   Figure 10 shows the % neutralisation of TGFβ2 activity by single chain Fv antibodies in an assay using proliferation of the erythroleukaemia cell line TF1 at different nM concentrations of scFv.

Figure 11 shows the neutralisation of TGFβ2 activity by whole IgG4 antibodies in an assay using proliferation of the erythroleukaemia cell line TF1 at different nM concentrations of antibody.

5   Figure 12 shows the effect of treatment of animals with antibodies on neural scarring as measured by the deposition of (Figure 12(a)) fibronectin and (Figure 12(b)) laminin detected using integrated fluorescence intensity. The graphs show scatter plots

10   of individual animal data points. The bar graph shows the mean integrated fluorescence intensity of the group.

Figure 13 shows the results of an ELISA to measure the cross-reactivity of the antibodies 6B1

15   IgG4 and 6A5 IgG4 with TGFβ isoforms and non-specific antigens. Figure 13(a) shows cross-reactivty of 6B1 IgG4 to a panel of non-specific antigens and TGFβ's, plotting OD405nm for each antigen: 1 - interleukin 1; 2 - human lymphotoxin (TNFβ); 3 - human insulin; 4 -

20   human serum albumin; 5 - ssDNA; 6 - oxazolone-bovine serum albumin; 7 - keyhole limpet haemocyanin; 8 - chicken egg white trypsin inhibitor; 9 - chymotrypsinogen; 10 - cytochrome C; 11 - GADPH; 12 - ovalbumin; 13 - hen egg lysozyme; 14 - bovine serum

25   albumin; 15 - TNFα; 16 - TGFβ1; 17 - TGFβ2; 18 - TGFβ3; 19 - PBS only. Figure 13(b) shows the OD405nm for the antibody 6A5 IgG4 against the same panel of antigens. For both Figure 13(a) and Figure 13(b),

antigens 1 to 15 were used for coating the plate at a concentration of 10μg/ml in PBS. The TGFbetas were coated at 0.2μg/ml in PBS. Coating was performed at 4°C overnight. 100μg of each antigen was used per

5 well and duplicates of each antigen for each IgG to be tested. IgG samples were incubated with the coated antigens at 37°C for 2 hours after blocking with 2% marvel-PBS. The labelled second antibody was a mouse anti-human Fc1 alkaline phosphatase conjugated and the

10 substrate used to detect bound second antibody was PNPP at 1mg/ml with the absorbance read at 405nm.

Figure 14 shows the amino acid and encoding nucleic acid sequence for the VL domain of the TGFβ1-specific antibody CS37.

15 Figure 15 shows data from an ELISA detecting binding of 6B1 IgG4 to BSA conjugated with either peptide TGF$\beta2_{56-69}$ or peptide TGF$\beta1_{56-69}$ coated on to an ELISA plate. 6B1 IgG4 was incubated at various concentrations in μg/ml and the absorbance at 405nm

20 measured after addition of the detection agents. OD405nm results are plotted at the various concentrations for BSA-TGF$\beta2_{56-69}$ ("Beta2 peptide" - diamonds) and BSA-TGF$\beta1_{56-69}$ ("Beta1 peptide" - squares).

25 Figure 16 shows % neutralisation of TGF-β2 anti-proliferative effect on TF1 cells by whole antibodies, 6H1 IgG4, 6B1 IgG4 and the mouse monoclonal from Genzyme, at various concentrations (nM IgG).

Figure 17 shows % neutralisation of TGF-β1 anti-proliferative effect on TF1 cells by whole antibodies,6H1 IgG4, 6B1 IgG4 and the mouse monoclonal from Genzyme, at various concentrations (nM IgG).

5 Figure 18 shows % neutralisation of TGF-β3 anti-proliferative effect on TF1 cells by whole antibodies, 6H1 IgG4, 6B1 IgG4 and the mouse monoclonal from Genzyme, at various concentrations (nM IgG).

Figure 19 shows amino acid and encoding DNA

10 sequences of regions of antibodies directed against TGFβ2 showing CDR sequences in italics: Figure 19(i) 2A-H11 VH (also known as 6H1 VH); Figure 19(ii) 6B1 VL; Figure 19(iii) 6A5 VL and Figure 19(iv) 6H1 VL.

Figure 20 shows the vector p6H1 VH-gamma4 (7263

15 bp). The gene encoding 6H1 VH is inserted as a HindIII-ApaI restriction fragment.

Figure 21 shows the vector p6B1 lambda (10151 bp). The gene encoding 6B1 VL is inserted as an EcoRI-BstBI restriction fragment.

20 Figure 22 shows the vector p6B1 gamma4gs (14176 bp). The genes encoding the heavy and light chains of 6BI IgG4 are combined in a single vector.

Figure 23 shows the results of competition ELISA experiments described in Example 6. Following

25 overnight incubation with TGFβ2, plates were treated with the following solutions 1-4 (number corresponding to those in Figure): 1 - 400µl Hams F12/DMEM (reagent blank), 2 - 400µl Hams F12/DMEM plus 4µg 6B1 IgG4

antibody (positive control), 3 - 400μl PC3 untreated

conditioned media plus 4μg 6B1 IgG4 antibody (latent

TGFβ$_2$ sample), 4 - 400μl PC3 acid activated

conditioned media plus 4μg 6B1 IgG4 antibody (active

5   TGFβ$_2$ sample).

All documents mentioned herein are incorporated

by reference.

10   **List of Examples**

Example 1 - Isolation of antibodies specific for

TGFβ1, antibodies specific for TGFβ2 and antibodies

specific for TGFβ1 and TGFβ2.

15   Example 2 - Construction of cell lines expressing

whole antibodies.

Example 3 - Neutralisation of TGFβ activity by

antibodies assessed using in vitro assays.

Example 4 - Inhibition by antibodies of TGFβ

20   binding to receptors.

Example 5 - Prevention of neural scarring using

antibodies against TGFβ.

Example 6 - Determination of Binding of 6B1 IgG4

to Active or Latent Form of TGFβ$_2$.

25   Example 7 - Neutralisation by antibodies directed

against TGFβ2 of the inhibitory effect of TGFβ

isoforms on cells proliferation.

Example 8 - Inhibition by antibodies directed

against TGFβ2 of binding of other TGFβ isoforms to receptors measured in a radioreceptor assay.

Example 9 - Assessment of TGFβ1 antibodies for potential therapeutic use.

5   Example 10 - Construction of a high expressing cell line for 6B1 IgG4 using the glutamine synthase selection system and assessment in a neutralisation assay.

Example 11 - Determination of the epitope on

10   TGFβ2 for the antibody 6B1 using a peptide phage display library.

Example 12 - Determination of the binding of 6B1 IgG4 to tissues by immunocytochemistry (ICC).

Example 13 - Determination of the kinetic

15   parameters of 6B1 IgG4 and single chain Fv for binding to TGFβ2.

Example 14 - Binding of a Peptide Corresponding to Residues 56 to 69 of TGFβ2 to 6B1 IgG4.

20   *EXAMPLE 1*

Isolation and Characterisation of Antibodies Binding to TGFβ1 and TGFβ2

<u>1 Identification and Characterisation of Antibodies to</u>

25   <u>Human TGFb-1 by Selection of Naive and Synthetic Phage</u>
<u>Antibody Repertoires</u>

Antibody repertoires

The following antibody repertoires were used:

1. <u>Peripheral blood lymphocyte (PBL) library</u> derived

from unimmunized human (Marks, J. D., Hoogenboom, H.

5   R. Bonnert, T. P., McCafferty, J., Griffiths, A. D. &

Winter, G. (1991) J. Mol. Biol. 222, 581-597)

2. <u>Synthetic library</u> (Nissim, A., Hoogenboom, H. R.,

Tomlinson, I. M., Flynn, G., Midgley, C., Lane, D. and

10   Winter, G. (1994) EMBO J. 13, 692-698) derived from

cloned human germline VH genes and synthetic CDR3s

with a fixed light chain

3. <u>Tonsil library</u> derived from the tonsils of

15   unimmunised humans. Tonsil B cells were isolated from

freshly removed (processed within 2 hours) whole

tonsils provided by Addenbrookes Hospital, Hills Road,

Cambridge, U.K. Each tonsil was processed as follows.

Tonsils were placed in a petri dish containing 5ml of

20   PBS and macerated with a scalpel blade to release the

cells. The suspension was transferred to a fresh tube

and large debris allowed to sediment under gravity for

5 minutes. The cell suspension was then overlaid onto

10mls of Lymphoprep in a 50 ml polypropylene tube

25   (Falcon) and centrifuged at 1000xg 20 minutes at room

temperature (no brake) and cells at the interface

harvested with a glass pipette. These were diluted to

a final volume of 50 ml in RPMI medium at 37^0 C and

centrifuged at 500xg for 15 minutes at room temperature. The supernatant was aspirated and the the cells washed another two times with RPMI.

Polyadenylated RNA was prepared from pelleted cells using the "QuickprepTM mRNA Kit" (Pharmacia Biotech, Milton Keynes, U.K.). The entire output of cells from one tonsil (ca. $1x10^6$ cells) was processed using one Oligo(dT)-Cellulose Spun column and processed exactly as described in the accompanying protocol. MRNA was ethanol precipitated as described and resuspended in 40ml RNase free water.

The cDNA synthesis reaction was set up using the "First-Strand cDNA Synthesis Kit (Pharmacia Biotech, Milton Keynes, U.K.) as follows:

RNA	$20\mu l$ (heated to 67°C 10 minutes before use)
1st strand buffer	$11\mu l$
DTT solution	$1\mu l$
pd(N)$_6$ primer	$1\mu l$

After gentle mixing, the reaction was incubated at 37°C for 1 hour.

Human VH genes were amplified from tonsil cDNA using the nine family-based back primers (VH 1b/7a -6a back *Sfi* , which introduce a *Sfi* I site at the 5'-end, Table 1) together with an equimolar mixture of the four JH forward primers (JH 1-2, 3, 4-5, 6, for; Marks et al., 1991 supra). Thus, nine primary PCR amplifications were performed. Each reaction mixture

(50 μl) comprised 2 μl cDNA template, 25 pmol back

primer, 25 pmol forward primers, 250 μM dNTPs, 1.5 mM

$MgCl_2$, 50 mM KCl, 10 mM Tris-HCL pH 8.3 and 2.5 u of

Taq polymerase (Boehringer). The reaction mixture was

5   overlaid with mineral (paraffin) oil and was cycled 30

times (94 ^{0}C for 1 min, 55 ^{0}C for 1 min, 72 ^{0}C for 1

min) using a Techne thermal cycler. The products were

purified on a 1% (w/v) agarose gel, isolated from the

gel using "Geneclean" (Bio 101 Inc.) and resuspended

10   in 15 μl of water. The amplified VH genes were

recombined with human VL genes derived from PBLs

(Marks et al., 1991 supra) together with the $(Gly_4,$

$Ser)_3$ linker (Huston, J.S., et al. 1988 Proc Natl Acad

Sci U S A. 85: 5879-83) by PCR assembly (Marks et al,

15   1991 supra). The VH-linker-VL antibody constructs were

cloned into the SfiI and NotI sites of the phagemid

vector, pCANTAB6 (McCafferty, J., et al. 1994 Appl.

Biochem. Biotech. 47: 157 - 173) to give a library of

6 x 10^7 clones.

20

4. <u>Large single chain Fv library</u> derived from lymphoid

tissues including tonsil, bone marrow and peripheral

blood lymphocytes.

     Polyadenylated RNA was prepared from the B-cells

25   of various lymphoid tissues of 43 non-immunised donors

using the "Quickprep mRNA Kit" (Pharmacia).

First-strand cDNA was synthesized from mRNA using a

"First-strand cDNA synthesis" kit (Pharmacia) using

random hexamers to prime synthesis. V-genes were
amplified using family-specific primers for VH, Vκ and
Vλ genes as previously described (Marks et al., supra)
and subsequently recombined together with the (Gly$_4$,

5   Ser)$_3$ scFv linker by PCR assembly. The VH-linker-VL
antibody constructs were cloned into the Sfi I and Not
I sites of the phagemid vector, pCANTAB 6. Ligation,
electroporation and plating out of the cells was as
described previously (Marks et al, 1991 supra). The

10   library was made ca. 1000x larger than that described
previously by bulking up the amounts of vector and
insert used and by performing multiple
electroporations. This generated a scFv repertoire
that was calculated to have ca. 1.3 x 10^{10} individual

15   recombinants which by Bst NI fingerprinting were shown
to be extremely diverse.

a. *Induction of phage antibody libraries*

      The four different phage antibody repertoires
20   above were selected for antibodies to TGFβ-1. The VH
synthetic (Nissim et al., 1994 supra), tonsil, 'large'
scFv and PBL (Marks et al., 1991 supra) repertoires
were each treated as follows in order to rescue
phagemid particles. 500 ml prewarmed (37 ^{0}C) 2YTAG

25   (2YT media supplemented with 100 μg/ml ampicillin and
2 % glucose) in a 2 l conical flask was inoculated
with approximately 3 x 10^{10} cells from a glycerol stock
(-70 ^{0}C) culture of the appropriate library. The

culture was grown at 37 ^{0}C with good aeration until the OD$_{600nm}$ reached 0.7 (approximately 2 hours). M13K07 helper phage (Stratagene) was added to the culture to a multiplicity of infection (moi) of

5 approximately 10 (assuming that an OD$_{600nm}$ of 1 is equivalent to 5 x 10^8 cells per ml of culture). The culture was incubated stationary at 37 ^{0}C for 15 minutes followed by 45 minutes with light aeration (200 rpm) at the same temperature. The culture was

10 centrifuged and the supernatant drained from the cell pellet. The cells were resuspended in 500 ml 2YTAK (2YT media supplemented with 100 μg/ml ampicillin and 50 μg/ml kanamycin), and the culture incubated overnight at 30 ^{0}C with good aeration (300 rpm).

15 Phage particles were purified and concentrated by three polyethylene glycol (PEG) precipitations (Sambrook, J., Fritsch, E.F., & Maniatis, T. (1990). Molecular Cloning - A Laboratory Manual. Cold Spring Harbour, New York) and resuspended in PBS to 10^{12}

20 transducing units (tu)/ml (ampicillin resistant clones).

b. Panning of phage antibody library on TGFβ-1

Phage induced from the four repertoires were each

25 separately panned on TGFβ-1. A 75mm x 12mm immuno tube (Nunc; Maxisorp) was coated with 2 ml of recombinant human TGFβ-1 (0.5ug/ml, Genzyme) in PBS overnight at 4 ^{0}C. After washing 3 times with PBS, the tube was

filled with 3%MPBS (3 % 'Marvel' skimmed milk powder, 1x PBS) and incubated for 2 hours at 37 ^{0}C for blocking. The wash was repeated, phagemid particles (10^{13} tu) in 2 ml of 3% MPBS were added and the tube

5 incubated stationary at 37 ^{0}C for 1 hour. The tube was washed 20 times with PBST(0.1%), then 20 times with PBS. Bound phage particles were eluted from the tube by adding 2 ml of 100mM-triethylamine, and incubating the tube stationary at room temperature for

10 10 minutes. The eluted material was immediately neutralised by pipetting into a tube containing 1 ml 1M-Tris.HCl (pH7.4). Phage were stored at 4 ^{0}C. 1.5 ml of the eluted phage were used to infect 20 ml of logarithmically growing E. coli TG1 (Gibson, T.J.

15 (1984). PhD thesis. University of Cambridge, UK.). Infected cells were grown for 1 hour at 37 ^{0}C with light aeration in 2YT broth, and then plated on 2YTAG medium in 243mm x 243mm dishes (Nunc). Plates were incubated overnight at 30 ^{0}C. Colonies were scraped

20 off the plates into 10 ml of 2YT broth and 15 % (v/v) glycerol added for storage at -70 ^{0}C.

Glycerol stock cultures from the first round of panning of each of the four repertoires on TGFβ-1 were each rescued using helper phage to derive phagemid

25 particles for the second round of panning. 250 μl of glycerol stock was used to inoculate 50 ml 2YTAG broth, and incubated in a 250 mL conical flask at 37 ^{0}C with good aeration until the OD$_{600mn}$ reached 0.7

(approximately 2 hours). M13K07 helper phage (moi=10) was added to the culture which was then incubated stationary at 37 ^{0}C for 15 minutes followed by 45 minutes with light aeration (200 rpm) at the same

5 temperature. The culture was centrifuged and the supernatant drained from the cell pellet. The cells were resuspended in 50 ml prewarmed 2YTAK, and the culture incubated overnight at 30 ^{0}C with good aeration. Phage particles were purified and

10 concentrated by PEG precipitation (Sambrook et al., 1990 supra) and resuspended in PBS to 1013 tu/ml.

Phage induced from the first round of panning of each of the three repertoires, was selected a second time essentially as described above except that the

15 panning tube was coated with only 1 ml of TGFβ-1 (0.5ug/ml, Genzyme), and the volume of phage added to the tube similarly reduced. After extensive washing, bound phage were eluted from the tube using 1 ml of 100 mM-triethylamine, and neutralised by the addition

20 of 0.5 ml 1M-Tris.HCl (pH7.4) as earlier described. The process of phage growth and panning was repeated over a third and a fourth round of selection.

c. Growth of single selected clones for immunoassay

25 Individual colonies from the third and fourth round selections were used to inoculate 100 μl 2YTAG into individual wells of 96 well tissue culture plates (Corning). Plates were incubated at 30 ^{0}C overnight

with moderate shaking (200 rpm). Glycerol to 15 % was added to each well and these master plates stored at -70 °C until ready for analysis.

5   *d. ELISA to identify anti-TGFβ-1 scFv*

Clones specific for TGFβ-1 were identified by ELISA, using scFv displayed on phage or soluble scFv.

i. Phage ELISA

10   Cells from the master plates were used to inoculate fresh 96 well tissue culture plates containing 100 μl 2YTAG per well. These plates were incubated at 37 °C for 6-8 hours or until the cells in the wells were growing logarithmically (OD600

15   0.2-1.0). M13K07 was added to each well to an moi of 10 and incubated stationary for 15 min then 45 min with gentle shaking (100 rpm), both at 37 °C. The plates were centrifuged at 2000 rpm for 10 min and the supernatant eluted. Each cell pellet was resuspended

20   in 100 μl 2YTAK and incubated at 30 °C overnight.

Each plate was centrifuged at 2000 rpm and the 100 μl supernatant from each well recovered and blocked in 20 μl 18%M6PBS (18 % skimmed milk powder, 6 x PBS), stationary at room temperature for 1 hour.

25   Meanwhile, flexible microtitre plates which had been blocked overnight stationary at 4 °C with either 50 μl 0.2 μg/ml TGFβ-1 in PBS or 50 μl PBS alone (giving an uncoated control plate), were washed 3 times in PBS

and blocked for 2 h stationary at 37 ^{0}C in 3MPBS.

These plates were then washed three times with PBS and

50 μl preblocked phage added to each well of both the

TGFβ-1-coated or uncoated plate.   The plates were

5   incubated stationary at 37 ^{0}C for 1 h after which the

phage were poured off.   The plates were washed by

incubating for 2 min in PBST three times followed by

incubating for 2min in PBS three times, all at room

temperature.

10   To each well of both the TGFβ-1-coated and the

uncoated plate, 50 μl of a 1 in 10,000 dilution of

sheep anti-fd antibody (Pharmacia) in 3MPBS was added

and the plates incubated at 37 ^{0}C stationary for 1 h.

Each plate was washed as described above and 50 μl of

15   a 1 in 5,000 dilution donkey anti-sheep alkaline

phosphatase conjugate (Sigma) in 3MPBS added and

incubated stationary at 37 ^{0}C for 1 h.   Plates were

washed as described as above followed by two rinses in

0.9% NaCl.   Alkaline phosphatase activity was

20   visualised using either the chromagenic substrate pNPP

(Sigma) or the Ampak system (Dako).   The absorbance

signal generated by each clone was assessed by

measuring the optical density at either 405 nm (pNPP)

or 492 nm (Ampak) using a microtitre plate reader.

25   Clones were chosen for further analysis if the ELISA

signal generated on the TGFβ-1-coated plate was at

least double that on the uncoated plate.

ii. Soluble ELISA

Cells from the master plates were used to inoculate fresh 96 well tissue culture plates containing 100 μl 2YTAG per well. These plates were
5   incubated at 30 ^{0}C for 8 hours then centrifuged at 2000 rpm for 10 min and the supernatant eluted. Each cell pellet was resuspended in 100 μl 2YTA (2YT media supplemented with 100ug/ml ampicillin) containing 10 mM IPTG (isopropyl-B-D-thiogalactopyranoside) and
10   incubated at 30 ^{0}C overnight.

Each plate was centrifuged at 2000 rpm and the 100 μl supernatant from each well recovered and blocked in 20 μl 18%M6PBS stationary at room temperature for 1 hour. Meanwhile, flexible
15   microtitre plates which had been blocked overnight stationary at 4 ^{0}C with either 50 μl 0.2 μg/ml TGFβ-1 in PBS or 50 μl PBS alone, were washed 3 times in PBS and blocked for 2 h stationary at 37 ^{0}C in 3%MPBS. These plates were then washed three times with PBS and
20   50 μl preblocked soluble scFv added to each well of both the TGFβ-1-coated or uncoated plate. The plates were incubated stationary at 37 ^{0}C for 1 h after which the scFv solutions were poured off. The plates were washed by incubating for 2 min in PBST (PBS
25   containing 1% Tween) three times followed by incubating for 2 min in PBS three times, all at room temperature.

To each well of both the TGFβ-1-coated and the

uncoated plate, 50 µl of a 1 in 200 dilution of the anti-myc tag murine antibody 9E10 (Munro, S. & Pelham, H.R.B. (1986)Cell 46, 291-300) in 3MPBS was added and the plates incubated at 37 °C stationary for 1 h.

5 Each plate was washed as described above and 50 µl of a 1 in 5,000 dilution goat anti-mouse alkaline phosphatase conjugate (Pierce) in 3MPBS added and incubated stationary at 37 °C for 1 h. Plates were washed as described above followed by two rinses in 10 0.9% NaCl. Alkaline phosphatase activity was visualised using either the chromagenic substrate pNPP (Sigma) or the Ampak system (Dako). The absorbance signal generated by each clone was assessed by measuring the optical density at either 405 nm (pNPP) 15 or 492 nm (Ampak) using a microtitre plate reader. Clones were chosen for further analysis if the ELISA signal generated on the TGFβ-1-coated plate was at least double that on the uncoated plate.

20 iii. Specificity ELISA

Clones identified as binding TGFβ-1 rather an uncoated well, as described above, were further analysed for fine specificity. Specificity ELISA's were carried out using scFv either displayed on phage 25 or in solution as described above, except that 5 ml of media in 50 ml Falcon tubes were inoculated with each clone and grown to generate the phage or soluble scFv used in the ELISA. Microtitre plate wells were coated

with 50 µl of either 0.2 µg/ml TGFβ-1, 0.2 µg/ml

TGFβ-2, 10 µg/ml bovine serum albumin (BSA) or PBS

(the uncoated well). After preblocking both the phage

(or soluble scFv) and the microtitre plates, 50 µl

5   blocked phage (or soluble scFv) from each clone was

added to a well coated with either TGFβ-1, TGFβ-2,

BSA or an uncoated well. As above, alkaline

phosphatse activity was visualised using either the

chromagenic substrate pNPP (Sigma) or the Ampak system

10   (Dako). Clones were considered to be specific for

TGFβ-1 if the ELISA signal generated in the TGFβ-1

coated well was at least five-fold greater than the

signal on either TGFβ-2, BSA or an uncoated well.

15   iv. Specificity determination by BIACore™

The antibodies were also shown to be specific for

TGFβ1 compared to TGFβ2 (obtained from R&D Systems

Abingdon) by relative binding to theBIACore™ sensor

chips coated with the appropriate antigen. TGFβ1 and

20   TGFβ2 were immobilised by amine coupling to Biosensor

CM5 sensorchips (Pharmacia) according to the

manufacturers instructions. Single chain Fv fragments

(35µl; purified by immobilized metal affinity

chromatography as described in example 4) were

25   injected over the immobilized antigen at a flow rate

of 5µl/min. The amount of TGFβ bound was assessed as

the total increase in resonance units (RUs) over this

period. For 31G9 scFv an increase of 1059RUs was found

with a TGFβ1 chip and 72 RUs was found with a TGFβ2
chip. Thus binding is much stronger to TGFβ1 than
TGFβ2.

5   *e. Sequencing of TGFb1-Specific ScFv Antibodies*

The nucleotide sequence of the TGFβ-1 specific
antibodies was determined by first using
vector-specific primers to amplify the inserted DNA
from each clone. Cells from an individual colony on
10   a 2YTAG agar plate were used as the template for a
polymerase chain reaction (PCR) amplification of the
inserted DNA using the primers pUC19reverse and
fdtetseq (Table 1). Amplification conditions
consisted of 30 cycles of 94 ^{0}C for 1 min, 55 ^{0}C for 1
15   min and 72 ^{0}C for 2 min, followed by 10 min at 72 ^{0}C.
The PCR products were purified using a PCR Clean-up
Kit (Promega) in to a final volume of 50 µl H20.
Between 2 and 5 µl of each insert preparation was used
as the template for sequencing using the Taq
20   Dye-terminator cycle sequencing system (Applied
Biosystems). The primers mycseq10 and PCR-L-Link were
used to sequence the light chain of each clone and
PCR-H-Link and pUC19reverse to sequence the heavy
chain (Table 1)

25

*f. Sequence and Source of the Initial TGFβ-1-Specific
ScFv Antibodies*

Four different TGFβ-1 specific antibodies were

isolated from the selections using the four libraries described above. Each clone name, its origin and its heavy and light chain germline is given below. The complete sequence of the VH domain genes of clones 1-

5   B2 and 31-G9 are given in Figure 1(a) together with the VL domain gene from scFv 31-G9.

CLONE	LIBRARY SOURCE	VH GERMLINE		VL ISOTYPE
1-B2	PBL	VH3	DP49	VKappa
1A-E5	Synthetic VH	VH3	DP53	VLambda
1A-H6	Tonsil	VH3	DP50	VLambda
31-G9	large scFv	VH3	DP49	VLambda

15   Thus these initial isolates were obtained from libraries derived from different sources-both natural V genes of unimmunised humans and synthetic libraries from cloned germline V genes together with synthetic CDRs.

20

2. Affinity Maturation of the Initial TGFβ-1-Specific ScFv Antibodies

a. Light Chain Shuffling of the TGFβ-1-Specific ScFv
25   Antibody 1-B2

i. Construction of Repertoires

The heavy chain of clone 1-B2 was recombined with

the complete repertoire of light chains derived from the PBL and large (tonsil-derived) scFv repertoires. The 1-B2 heavy chain was amplified by PCR using the primers HuJh4-5For (Table 1) and pUC19reverse.

5 Amplification conditions consisted of 30 cycles of 94 ^{0}C for 1 min, 55 ^{0}C for 1 min and 72 ^{0}C for 1min, followed by 10 min at 72 ^{0}C. The PCR product was separated through a 1% agarose-TAE gel, the band representing the amplified VH excised, and eluted from

10 the agarose gel using the Geneclean Kit (Bio 101).

The PBL and tonsil light chains were amplified by PCR using the primers fdtetseq and a mix of RL1, 2 & 3 (Table 1). Amplification conditions consisted of 30 cycles of 94 ^{0}C for 1 min, 55 ^{0}C for 1 min and 72 ^{0}C

15 for 1min, followed by 10 min at 72 ^{0}C. The PCR product was separated through a 1% agarose-TAE gel, the band representing the amplified VL excised, and eluted from the agarose gel using the Geneclean Kit (Bio 101).

20 Approximately 50 ng amplified 1-B2 heavy chain and 50 ng of either amplified PBL-derived or amplified tonsil-derived light chains were combined and precipitated with sodium acetate and ethanol using 25 µg glycogen as a carrier. The precipitated DNA was

25 pelleted by centrifugation at 13,000 rpm in a microfuge, air dried and resuspended in 26 µl H20. This was used in an assembly amplification after the addition of reaction buffer to 1X, dNTP's to 200 nM

and 5 units Taq polymerase. Amplification conditions consisted of 20 cycles of 94 ^{0}C for 1 min, 60 ^{0}C for 1 min and 72 ^{0}C for 1min 30 s, followed by 10 min at 72 ^{0}C. 10 µl of each assembly was used as the template

5   in a 'pull-through' amplification with the primers fdtetseq and pUC19reverse. Amplification conditions consisted of 25 cycles of 94 ^{0}C for 1 min, 60 ^{0}C for 1 min and 72 ^{0}C for 1min 30 s, followed by 10 min at 72 ^{0}C.

10   The pull-through amplification product was separated through 1% agarose-TAE and the band representing the pull-through VH-VL excised and eluted using the Geneclean Kit. This was digested with the restriction endonucleases Sfi I and Not I (NEB) and

15   ligated (Amersham ligation system) into the phagemid vector pCantab 6, previously digested with Sfi 1 and Not I. The ligation product was used to transform electrocompetent TG1 cells, plated out on 2YTAG plates and incubated overnight at 30 ^{0}C. Approximately 1 x

20   10^5 individual clones were generated from the light chain-shuffle of the 1-B2 heavy chain with the PBL-derived light chains and approximately 1 x 10^6 for the shuffle with the tonsil-derived light chains.

25   ii. Selection of Light Chain Shuffle Repertoires
       The two light chain-shuffle repertoires were selected for TGFβ-1-specific antibodies. Phagemid particles were recovered from each repertoire as

described earlier for the initial libraries.
Recovered phage were preblocked for 1 h in a final
volume of 100 µl 3MPBS. Approximately 10^{11} tu phage
were used in the first round selection and between 10^9

5   and 10^{10} for subsequent selections. For the first
round selections, biotinylated TGFβ1 to a final
concentration of 100 nM was added to the preblocked
phage and incubated stationary at 37^0C for 1h.

For each selection, 100 µl Dynabeads suspension

10   (Dynal) was separated on a magnet and the beads
recovered and preblocked for 2 h in 1 ml 3MPBS. The
beads were recovered on a magnet and resuspended in
the phagemid/biotinylated TGFβ-1 mixture and incubated
at room temperature for 15 min while being turned

15   end-over-end. The beads were captured on a magnet and
washed four times with PBST followed by three washes
in PBS. After each wash, the beads were captured on a
magnet and resuspended in the next wash. Finally,
half of the beads were resuspended in 10 µl 50 mM DTT

20   (the other half of the beads stored at 4 ^{0}C as a
back-up) and incubated at room temperature for 5 min.
The whole bead suspension was then used to infect 5 ml
logarithmically-growing TG1 cells. This was incubated
at 37 ^{0}C, stationary for 15 min then with moderate

25   shaking for 45 min, plated on 2YTAG plates and
incubated overnight at 30 ^{0}C.

Colonies were scraped off the plates into 10 ml
of 2YT broth and 15 % (v/v) glycerol added for storage

at -70 ^{0}C. A 250 µl aliqout of each plate scrape was used to inoculate 2YTAG and phagemid particles rescued as described earlier. For each repertoire, three rounds of selection using biotinylated TGFβ-1 was

5   performed, essentially identical to the first round selection described above. All selections were at 100 nM TGFβ-1 except for the third round selection of the tonsil-derived light chain repertoire where the concentration of biotinylated TGFβ-1 in the selection

10   was reduced to 50 nM.

iii. Identification of TGFβ-1-Specific ScFv Antibodies from Light Chain Shuffle Repertoires

     ScFv antibodies specific to TGFβ-1 were

15   identified by both phage and soluble ELISA, and sequenced, as described earlier. Three new TGFβ-1-specific scFv antibodies were identified, two with PBL-derived light chains and one with a tonsil-derived light chain. All three had the 1B2

20   heavy chain sequence (DP49), described earlier. The sequences are summarised below and the complete sequence of each VL domain gene is given in figure 1(b).

CLONE	VL SOURCE	VH GERMLINE	VL ISOTYPE
7-A3	PBL	DP49 (1B2)	VKappa
10-A6	PBL	DP49 (1B2)	VLambda

14-A1   Tonsil   DP49 (1B2)   VLambda

　　　Thus the VH domain 1B2 derived from the PBL

library can be combined with VL domains derived from

5   both PBL and tonsil libraries.

*b. CDR3 'Spiking' of the TGFβ-1-Specific ScFv Antibody
1B2*

10   i. Construction of 'spiked' repertoire

　　　An 84 mer mutagenic oligonucleotide primer, 1B2

mutVHCDR3, was first synthesized (see Table 1). This

primer was 'spiked' at 10%; i.e. at each nucleotide

position there is a 10% probability that a

15   non-parental nucleotide will be incorporated. The

1-B2 heavy chain was amplified by PCR using the

primers pUC19reverse and 1B2 mutVHCDR3. Amplification

conditions consisted of 30 cycles of 94 ^{0}C for 1 min,

55 ^{0}C for 1 min and 72 ^{0}C for 1min, followed by 10 min

20   at 72 ^{0}C. The PCR product was separated through a 1%

agarose-TAE gel, the band representing the amplified

VH excised, and eluted from the agarose gel using the

Geneclean Kit (Bio 101).

　　　The parental 1B2 light chain was amplified by PCR

25   using the primers fdtetseq and RL3 (Table 1).

Amplification conditions consisted of 30 cycles of 94

^{0}C for 1 min, 55 ^{0}C for 1 min and 72 ^{0}C for 1min,

followed by 10 min at 72 ^{0}C. The PCR product was

separated through a 1% agarose-TAE gel, the band
representing the amplified VL excised, and eluted from
the agarose gel using the Geneclean Kit (Bio 101).

5   Approximately 50 ng amplified 'spiked' 1-B2 heavy
chain and 50 ng of amplified parental 1B2 light chain
were combined and precipitated with sodium acetate and
ethanol using 25 μg glycogen as a carrier. The
precipitated DNA was pelleted by centrifugation at
13,000 rpm in a microfuge, air dried and resuspended

10   in 26 μl H20. This was used in an assembly
amplification after the addition of reaction buffer to
1X, dNTP's to 200 nM and 5 units Taq polymerase.
Amplification conditions consisted of 25 cycles of 94
^{0}C for 1 min, 65 ^{0}C for 4 min. Five μl of each

15   assembly was used as the template in a 'pull-through'
amplification with the primers fdtetseq and
pUC19reverse. Amplification conditions consisted of
30 cycles of 94 ^{0}C for 1 min, 55 ^{0}C for 2 min and 72 ^{0}C
for 1min, followed by 10 min at 72 ^{0}C.

20   The pull-through amplification product was
separated through 1% agarose-TAE and the band
representing the pull-through 'spiked' VH -VL excised
and eluted using the Geneclean Kit. This was digested
with the restriction endonucleases Sfi I and Not I

25   (NEB) and ligated (Amersham ligation system) into the
phagemid vector pCantab 6, previously digested with
Sfi I and Not I. The ligation product was used to
transform electrocompetent TG1 cells, plated out on

2YTAG plates and incubated overnight at 30 ^{0}C.

Approximately 4 x 10^{6} individual clones were generated

from this VH CDR3 'spiking' of the 1-B2 VH CDR3.

5   ## ii. Selection of 1B2 CDR3 Spike Repertoire

The repertoire was selected for new

TGFβ-1-specific scFv antibody by one round of panning

on 1 μg/ml TGFβ-1 followed by two rounds of selection

with biotinylated TGFβ-1 at 50 nM using methods as

10   described earlier.

iii. Identification of TGFß-1-Specific ScFv Antibodies from the 1B2 CDR3 Spike Repertoire

ScFv antibodies specific to TGFβ-1 were

15   identified by both phage and soluble and phage ELISA,

and sequenced, as described earlier.   Clone 27C1 was

isolated from the spiked repertoire.   It is virtually

identical to clone 1B2 but with three differences in

the heavy chain CDR3. The complete sequence of clone

20   27C1 is given in figure 1 (c). The 27C1 VH domain was

combined with the 10A6 VL domain in the construction

of the whole antibody 27C1/10A6 IgG4 (example 2). The

properties of this antibody are described in more

detail in examples 2 to 6. In addition to 27C1, a

25   large number of other antibodies were isolated with up

to 7 of the 14 amino acids differing in CDR3 of the VH

domain (Figure 3). These had a similar preference for

binding TGFβ1 compared to TGFβ2.

3. Identification and Characterisation of Antibodies to Human TGFβ-2 by Selection of Naive and Synthetic Phage Antibody Repertoires

5   a. Induction of phage antibody libraries

Two different phage antibody repertoires were selected for antibodies to TGFβ-2. The VH synthetic (Nissim et al., 1994) and tonsil (constructed as described earlier) repertoires were each treated as

10   described for TGFβ-1 to rescue phagemid particles.

b. Panning of phage antibody library on TGFβ-2

Phage induced from the two repertoires were each separately panned on TGFβ-2 as described earlier for

15   TGFβ-1 but using 0.5 μg/ml TGFβ-2 as the coating antigen.

c. Identification and Sequencing of TGFβ-2-Specific ScFv Antibodies

20   Individual colonies from the third and fourth round selections were screened by both phage and soluble ELISA as described earlier for TGFβ-1 but using flexible microtitre plates coated with TGFβ-2 at 0.2 μg/ml rather than TGFβ-1. Clones were chosen for

25   further analysis if the ELISA signal generated on the TGFβ-2-coated plate was at least double that on the uncoated plate. For the specificity ELISA, as described earlier for TGFβ-1, clones were considered

to be specific for TGFβ-2 if the ELISA signal generated in the TGFβ-2 coated well was at least five-fold greater than the signal on either TGFβ-1, BSA or an uncoated well.

5

d. Sequence and Source of the Initial TGFβ-2-Specific ScFv Antibodies

Four different TGFβ-2 specific antibodies were isolated from the selections using the two libraries described above. Each clone name, its origin and its heavy and light chain germline is given below. The complete sequence of the VH domain genes of 2A-H11 and 2A-A9 are given in Figure 2 (a).

15

CLONE	LIBRARY SOURCE	VH GERMLINE	VL ISOTYPE
1-G2	Tonsil		
1-H6	Tonsil	DP49	
2A-H11	Synthetic VH	DP50	VLambda
2A-A9	Synthetic	DP46	VLambda
Gold-11	Large scFv		VLambda

Thus human antibodies binding to human TGFβ2 have been isolated from different sources-, both natural Vgenes of unimmunised humans and synthetic libraries from cloned germline V genes together with synthetic CDRs.

4. Light Chain Shuffling of the TGFβ-2-Specific ScFv
Antibodies 2A-H11 and 2A-A9

a. Construction of Repertoires

5   The heavy chain of clones 2A-H11 and 2A-A9 were
recombined with the complete repertoire of light
chains derived from the PBL and large (tonsil-derived)
scFv repertoires as described earlier for the
TGFβ-1-specific scFv antibody 1-B2.   Both repertoires
10   generated from the recombination with the PBL light
chain repertoire were approximately 1×10^5, those
generated from the recombination with the tonsil light
chain repertoire were approximately 1×10^6.

15 b. Selection of Light Chain Shuffle Repertoires

The light chain-shuffle repertoires were selected
for TGFβ-2-specific antibodies using biotinylated
TGFβ-2, as described earlier for the selection of the
TGFβ-1 light chain shuffle repertoires.   For all of
20   the first and second round selections, a
concentrartion of 100 nM biotinylated TGFβ-2 was used.
For the third round selection of the PBL-derived light
chain shuffle repertoire, biotinylated TGFβ-2 was used
at concentrations of 100 nM and 1 nM.   For the third
25   round selection of the tonsil-derived light chain
shuffle repertoire, biotinylated TGFβ-2 was used at a
concentration of 50 nM.

c. Identification of TGFβ-2-Specific ScFv Antibodies from Light Chain Shuffle Repertoires

ScFv antibodies specific to TGFβ-2 were identified by both phage and soluble ELISA, and sequenced, as described earlier. Five new TGFβ-2-specific scFv antibodies were identified. The sequences are summarised below and the complete sequence of each clone given in Figure 2 (b).

CLONE	VL SOURCE	VH GERMLINE	VL ISOTYPE
6-H1	PBL	DP50 (2A-H11)	VKappa
6-A5	PBL	DP50 (2A-H11)	VLambda
6-B1	PBL	DP50 (2A-H11)	VLambda
11-E6	PBL	DP46 (2A-A9)	VKappa
14-F12	Tonsil	DP46 (2A-A9)	VLambda

d. Specificity determination by ELISA

Clones identified as binding TGFβ-2 rather an uncoated well, as described above, were further analysed for fine specificity. Specificity ELISA's were carried out using scFv either displayed on phage or in solution as described above, except that 5 ml of media in 50 ml Falcon tubes were inoculated with each clone and grown to generate the phage or soluble scFv used in the ELISA. Microtitre plate wells were coated with 50 µl of either 0.2 µg/ml TGFβ-1, 0.2 µg/ml TGFβ-2, 10 µg/ml bovine serum albumin (BSA) or PBS

(the uncoated well). After preblocking both the phage (or soluble scFv) and the microtitre plates, 50 µl blocked phage (or soluble scFv) from each clone was added to a well coated with either TGFβ-1, TGFβ-2, BSA

5   or an uncoated well. As above, alkaline phosphatse activity was visualised using either the chromagenic substrate pNPP (Sigma) or the Ampak system (Dako). Clones were considered to be specific for TGFβ-2 if the ELISA signal generated in the TGFβ-2 coated well

10   was at least five-fold greater than the signal on either TGFβ-1, BSA or an uncoated well. Cross-reactivity with unrelated antigens was determined more extensively for anti-TGFβ2 antibody in whole antibody format, see example 2. The

15   cross-reactivity of 6B1 IgG4 and 6A5 IgG4 with TGFβ1 and TGFβ3 (obtained from R&D Systems, Abingdon) is also shown to be very low.

e. Specificity determination by BIACore™

20   The antibodies were also shown to be specific for TGFβ2 compared to TGFβ1 by relative binding to theBIACore sensor chips coated with the appropriate antigen. TGFβ1 and TGFβ2 were immobilised by amine coupling to Biosensor CM5 sensorchips (Pharmacia)

25   according to the manufacturers instructions. Single chain Fv fragments (35µl; purified by immobilized metal affinity chromatography) were injected over the immobilized antigen at a flow rate of 5µl/min. The

amount of TGFβ bound was assessed as the total

increase in resonance units (RUs) over this period.

For the single chain Fv fragments 6H1, 6A5 and 14F12,

these fragments gave a total of 686, 480 and 616 RUs

5 respectively for the TGFβ1 coated sensor chip and 77,

71 and 115 RUs respectively for the TGFβ2 coated chip.

5.   Building higher affinity anti TGFβ-1 biological

neutralisers

10

a. Recombining heavy chains derived from high affinity

anti- TGFβ1 scFv with light chains derived from anti

-TGFβ1 and anti-TGFβ2 scFv showing good properties

Antibodies derived by spiking CDR3 of the scFv

15 antibody 1-B2 (section 2b) bind TGFβ-1 with high

affinity. To improve the chance of obtaining high

affinity neutralising antibodies it was decided to

chain shuffle VHs derived from high affinity

anti-TGFβ-1 scFv with VLs derived from scFv clones

20 with promising properties and particularly with those

capable of neutralising the activity of TGFβ-2 in

vitro.

Heavy chains were amplified by PCR from the

repertoire of CDR3 spiked 1-B2 clones after selection

25 on TGFβ-1(section 2a.ii) using the primers

pUC19reverse and PCR-H-Link (Table 1). Amplification

conditions consisted of 30 cycles of 94 ^{0}C for 1 min,

55 ^{0}C for 1 min and 72 ^{0}C for 1min, followed by 10 min

at 72 ^{0}C.   The PCR product was separated through a 1%

agarose-TAE gel, the band representing the amplified

VH excised, and eluted from the agarose gel using the

Geneclean Kit (Bio 101).

5   Light chains were separately amplified by PCR

from each of the anti TGFβ-1 specific neutralisers (

7-A3, 10-A6 and 14-A1; section 2a.iii) and each of the

anti TGFβ-2 specific neutralisers (6H1, 6A5, 6B1, 11E6

and 14F12; section 4c) using the primers fdtetseq1

10   and PCR-L-Link (Table 1). The same PCR conditions

were used as described for theVH amplification. Each

VL PCR product was then separately purified through a

1% agarose-TAE gel as described above. Purified

products were finally mixed in approximately equimolar

15   amounts (as estimated from an analytical agarose gel)

to provide a VL 'pool'.

        Approximately 50 ng amplified heavy chains and 50

ng of amplified pooled light chains were combined and

precipitated with sodium acetate and ethanol using 25

20   µg glycogen as a carrier. The precipitated DNA was

pelleted by centrifugation at 13,000 rpm in a

microfuge, air dried and resuspended in 23 µl H20.

This was used in an assembly amplification after the

addition of reaction buffer, dNTP's to 200 nM and 5

25   units Taq polymerase. Amplification conditions

consisted of 20 cycles of 94 ^{0}C for 1 min, 55 ^{0}C for 1

min and 72 ^{0}C for 2 mins, followed by 10 min at 72 ^{0}C.

5 µl of assembly was used as the template in a 50ul

'pull-through' amplification with the primers fdtetseq and pUC19reverse. Amplification conditions consisted of 30 cycles of 94 ^{0}C for 1 min, 55 ^{0}C for 1 min and 72 ^{0}C for 2mins, followed by 10 min at 72 ^{0}C.

5   The pull-through amplification product was separated through 1% agarose-TAE and the band representing the pull-through VH-VL excised and eluted using the Geneclean Kit. This was digested with the restriction endonucleases Sfi I and Not I (NEB) and

10   ligated into the phagemid vector pCantab 6 (McCafferty et al. 1994 supra), previously digested with Sfi 1 and Not I, using the Amersham ligation system. The ligation product was used to transform electrocompetent TG1 cells, plated out on 2YTAG plates

15   and incubated overnight at 30 ^{0}C. A repertoire of approximately 3 x 10^6 individual clones was generated.

b. Selection of chain shuffled repertoire

    The chain shuffled repertoire was selected by a

20   single round of panning on TGFβ-1 (1ug/ml), as previously described (section 1b).

c. Identification of TGFβ-1 specific scFv antibodies

    ScFv antibodies specific to TGFβ-1 were

25   identified by phage ELISA and sequenced as described earlier (sections 1d.i and 1e). New TGFβ-1 specific scFv antibodies were identified. Five new high affinity clones were isolated - CS32 which comprises

31G9 VH and 7A3 VL; CS39 which comprises 31G9 VH and
6H1 VL; CS37 which comprises 31G9 VH Figure 1(a) (iii)
and 11E6 VL with an Ile for Val substitution at
residue 2 (VL sequence given in Figure 14); CS35 which
5   comprises 31G9 heavy chain with substitutions of Glu
for Gln at residue 1, Gln for Glu at residue 5 and
14F12 VL; and CS38 which comprises 31G9 VH with
substitutions of Thr for Gln at residue 3, Glu for Gln
at residue 5, Leu for Phe at residue 27, Ile for Asn
10   at residue 56 and Arg for Gln at residue 105 and 6A5
VL.

d. Off-rate determination for single chain Fv fragments binding to TGFβ1 and TGFβ2

15   The off-rates for binding to TGFβ1 or TGFβ2 of
the single chain Fv fragments described in this
example were determined as described by Karlsson et al
(R. Karlsson et al, J. Immunol. Methods 145, 229-240,
1991). The results obtained are shown in Table 2,
20   together with dissociation constants for those which
have been determined. These results indicate that high
affinity antibodies have been isolated.

6. Identification and Characterisation of an Antibody
25   *which Cross-reacts with both Human TGFβ-1 and TGFβ-2*
but not TGFβ-3 by Selection of a Large ScFv Repertoire

a. Panning of the Library and Identification of

<u>Binders</u>

The large scFv library (described earlier) was induced, phagemid particles rescued and panned as described earlier with the following modifications.

5   For the first round of panning, 10^{12} tu library phage in 0.5 ml PBS were used (rather than the standard 2 ml), for the second round, 3.5×10^9 phage in 0.5 ml PBS were used. The immuno tube was coated with 10 µg TGFβ-2 in 0.5 ml PBS for both the first and second

10   round of selection. Individual colonies from the second selection were screened by ELISA using 0.2 µg/ml TGFβ-1. Clones binding TGFβ-1 were further screened on TGFβ-2, TGFβ-3, BSA and PBS. Clones were considered to be specific for both TGFβ-1 and TGFβ-2

15   if the ELISA signal generated in the TGFβ-1 and the TGFβ-2 coated wells were both at least five-fold greater than the signal on TGFβ-3, BSA and an uncoated well.

20   <u>c. Identification of a TGFβ-1/TGFβ-2 Cross-reactive ScFv Antibody</u>

A single scFv antibody specific for both TGFβ-1 and TGFβ-2 was identified by both phage and soluble ELISA, and sequenced, as described earlier. The

25   complete sequence of the VL domain of the antibody gene VT37 is given in figure 4. The dissociation constant of this single chain Fv antibody was estimated by analysis using BIACore™ to be 4nM for

TGFβ1 and 7nM for TGFβ2. Cross-reactivity for TGFβ3 was also determined. Purified VT37scFv at 8.3μg/ml was passed over BIACore™ sensor chips coated with TGFβ1 (500RUs coated); TGFβ2 (450RUs coated) or TGFβ3

5   (5500RUs coated). The relative response for VT37 scFv binding was: TGFβ1 – 391RU bound; TGFβ2 – 261RU bound or TGFβ3 – 24RU bound. Thus this antibody binds strongly to TGFβ1 and TGFβ2 but binding to TGF β 3 is not detectable above background.

10

EXAMPLE 2

Construction of Cell Lines Expressing Whole Antibodies

For the construction of cell lines expressing IgG4 antibodies, variable domains were cloned into

15   vectors expressing the human gamma 4 constant region for the VH domains or the human kappa or lambda constant regions for the VL domains.

To construct the whole antibody, 27C1/10A6 IgG4 (specific for TGFβ$_1$), 27C1 VH DNA was prepared from

20   the clone isolated above, in example 1. The VH gene was amplified by PCR using the oligonucleotides VH3BackSfiEu and VHJH6ForBam (Table 1) with cycles of 1 min at 94°C, 1 min at 55°C, 1.5 min at 72°C. Following digestion with SfiI and BamHI, the VH gene

25   was cloned into the vector vhcassette2 (Figure 5) digested with SfiI and BamHI. Ligated DNA was transformed into E. coli TG1. Ampicillin resistant colonies were obtained and those containing the

correct insert identified by DNA sequencing.

Plasmid DNA from these colonies was prepared and
the DNA digested with HindIII and BamHI. The
HindIII-BamHI restriction fragment was ligated into
5   the human IgG4 heavy chain expression vector pG4D100
(Figure 6), which had been digested with HindIII and
BamHI and the DNA transfected into E.coli TG1 by
electroporation. The sequence of the VH gene insert
was again verified by DNA sequencing.

10   For the light chain, the VL gene of 10A6,
isolated in example 1, was first mutagenized to remove
its internal BamHI site using site directed
mutagenesis (Amersham RPN1523) with the
oligonucleotide DeltaBamHI (Table 1). The resulting
15   VLDBamH1 gene was amplified by PCR using the
oligonucleotides Vλ3/4BackEuApa and HuJλ2-3ForEuBam
(Table 1). Following digestion of the amplified insert
with ApaLI and BamHI, the VL gene was cloned into the
vector vlcassetteCAT1 (Figure 7) digested with ApaLI
20   and BamHI. Ligated DNA was transformed into E.coli
TG1. Ampicillin resistant colonies were obtained and
those containing the correct insert were identified by
DNA sequencing.

Plasmid DNA from these colonies was prepared and
25   the DNA digested with Hind III and BamHI. The
HindIII-BamHI restriction fragment containing the
leader sequence and the VL domain was ligated into the
human lambda light chain expression vector, pLN10

(Figure 8), which had been digested with HindIII and BamHI. Following electroporation, transformants in E.coli were checked by DNA sequencing.

5 Plasmid DNA was prepared from the pG4D100-27C1 clone and the pLN10-10A6 clone. This DNA was then co-transfected into DUKXB11 Chinese Hamster Ovary (CHO) cells by electroporation (290V; 960μF). The cells were then grown for 2 days in non-selective medium (alpha-MEM plus nucleosides). Cells were then 10 transferred to a selective medium (alpha-MEM plus 1mg/ml G418 without nucleosides) and grown in 96 well plates. Colonies were then transferred to 24 well plates. and samples assayed by sandwich ELISA for assembled human IgG4 antibody and by binding to TGFβ1 15 in ELISA (as in example 1). For the sandwich ELISA, goat anti-human IgG coated on to the ELISA plate and captured human IgG4 detected using goat antihuman lambda light chain alkaline phosphatase conjugate. High expressing cell lines were then derived by 20 amplification of the inserted genes using selection in the presence of methotrexate (R.J. Kaufman Methods Enzymol. 185 537-566, 1990).

The whole antibody 6H1 IgG4 (specific for TGFβ2) was constructed in a similar way to the above 25 construction of 27C1/10A6 IgG4. The 6H1 VH gene (example 2) was cloned into pG4D100 as for 27C1 above except that PCR amplification was performed with the oligonucleotides VH3BackSfiEu and VHJH1-2FORBam. The

6H1 VL gene (example 2) was subcloned into

vlcassetteCAT1 as above except that PCR amplification

was performed with the oligonucleotides Vk2BackEuApa

and HuJk3FOREuBam. However, since the 6H1 VL is a

5   kappa light chain the HindIII-BamHI fragment was

subcloned into the human kappa light chain expression

vector pKN100 (Figure 9) which had been digested with

HindIII and BamHI. High expressing cell lines were

then isolated as described above. Clones expressing

10   antibody were identified from culture plates by

sandwich ELISA for assembled human IgG4 antibody

(detected using goat anti-human kappa light chain

conjugate and by binding to TGFβ2 in ELISA (as in

example 2).

15   To construct the whole antibodies 6A5 IgG4 and

6B1 IgG4, the same 6H1 VH construct in pG4D100 was

used as for 6H1IgG4 since these antibodies all have

the same VH gene. The 6B1 and 6A5 genes were each

subcloned into vlcassetteCAT1 as above for the 10A6

20   light chain except that PCR amplification was

performed with the nucleotides Vλ3backEuApa and

HuJλ2-3ForEuBam. The HindIII-BamHI restriction

fragment was then subcloned into pLN10 as above.

Clones expressing antibody were identified from

25   culture plates by sandwich ELISA for assembled human

IgG4 antibody (detected using goat anti-human kappa

light chain conjugate and by binding to TGFβ2 in ELISA

(as in example 2).

Properties of whole antibody constructs

Purification of whole antibodies

Serum-free supernatant from CHO cells producing
5   the relevant IgG was clarified by centrifugation at
8000 rpm (Beckman JS2-21) prior to purification. The
supernatant was applied to a HiTrap Protein A
Sepharose prepacked affinity column from Pharmacia,
either 1 or 5ml size, with binding capacities of 25 or
10   120 mg respectively. Each IgG had a dedicated column
to avoid any potential carry over of material from one
purification to another. The column was equilibrated
to phosphate buffered saline (PBS) with ten column
volumes of 1xPBS prior to applying the supernatant.
15   When all the supernatant had been applied to the
column at a flow rate of 2-4 ml/minute, again,
depending on the column size, the column was washed
with ten column volumes of 1xPBS to remove any
non-specifically bound material. Elution of the bound
20   protein was achieved using 0.1M sodium acetate,
adjusted to pH 3.3 with glacial acetic acid. The
eluted material was collected in 8 fractions of 1.5 ml
volume, and the amount of protein determined by
measuring the absorbance at 280nm, and multiplying
25   this value by 0.7 to get a value in mg/ml. This was
then neutralised with 0.5ml of 1M Tris.HCl pH 9.0 per
1.5ml fraction, and the protein-containing fractions
pooled and dialysed against 1x PBS to buffer exchange

the IgG.   The column was returned to neutral pH by
running ten column volumes of 1xPBS through, and was
stored in 20% ethanol as a preservative until required
again.

5.   A sample was then run on 10-15% SDS-PAGE (Phast
system, Pharmacia) and silver stained.   this allowed
an assessment of the purity of the IgG preparation.
This was usually found to be about 80-90%, with only a
couple of other bands prominent on the stained gel.

10

Binding specificity by ELISA

        The IgG4 antibodies 6B1 and 6A5 were shown to
bind TGFβ2 with very low cross-reactivity to TGFβ1 and
TGFβ3 and no detectable cross-reactivity with a range
15   of non-specific antigens: interleukin-1; human
lymphotoxin (TNFb); human insulin; human serum
albumin; single stranded DNA; oxazolone-bovine serum
albumin; keyhole limpet haemocyanin; chicken egg white
trypsin inhibitor; chymotrypsinogen; cytochrome c;
20   glyceraldehyde phosphate dehydrogenase; ovalbumin; hen
egg lysozyme; bovine serum albumin and tumour necrosis
factor a - (TNFa) (Figure 13(a) and (b)). Likewise the
antibodies 6B1, 6A5 and 6H1 IgG4 bound strongly to
TGFβ2 coated on a BIACore™ sensor chip but not
25   significantly to TGFβ1 or TGFβ3 coated chips.

Binding properties of whole antibodies by BIACore™
        The affinity constants of the above antibodies

were determined by BIACore™, using the method of
Karlsson et al. J. Immunol. Methods 145, 299-240, 1991
(supra) and found to be approximately 5nM for
27C1/10A6 IgG4 for TGFβ1 and 2nM for 6H1 IgG4 for

5   TGFβ2. The antibody 27C1/10A6 IgG4 also shows some
cross-reactivity with TGFβ2 coated onto Biosensor
chips but the dissociation constant is approximately
10 fold or more higher for TGFβ2 compared to TGFβ1.
There was no significant cross-reactivity with

10   lysozyme coated onto a BIACore™ sensor chip.

Neutralisation and inhibition of radioreceptor
binding by IgG4 antibodies to TGFβ1 and TGFβ 2 is
described in examples 3 and 4.

15   *EXAMPLE 3*

*Neutralisation by Antibodies of the Inhibitory Effect
of TGF β1 and TGF β2 on Cell Proliferation*

The neutralising activity of the antibodies
described in examples 1 and 2 were tested in a

20   modification of a bioassay for TGF β as described by
Randall et al (1993) J. Immunol Methods 164, 61-67.
This assay is based on the ability of TGF $β_1$ and TGF $β_2$
to inhibit the interleukin-5 induced proliferation of
the erythroleukaemia cell line, TF1 and being able to

25   reverse this inhibition with specific TGF β
antibodies.

Method

Cells and maintenance

The human erythroleukaemia cell line TF1 was grown in RPMI 1640 medium supplemented with 5% foetal calf serum, penicillin/streptomycin and 2ng/ml
5   rhGM-CSF in a humidified incubator containing 5% CO_2 at 37°C. Cultures were passaged when they reached a density of 2×10^5/ml and diluted to a density of 5×10^5/ml.

10   Cytokines and Antibodies

rhGM-CSF and rhIL-5 were obtained from R&D systems, rhTGF β_2 was obtained AMS Biotechnology. Rabbit anti TGF β_2 antibody was from R&D Systems and Mouse anti-TGF $\beta_{1,2,3}$ was from Genzyme. Other
15   antibodies against TGF β_2 were as described in examples 1&2.

Titration of Inhibition of Proliferation by TGF β_2.

Doubling dilutions of TGF β_2 (800pM - 25pM) for
20   the construction of a dose response curve were prepared on a sterile microtitre plate in 100µl of RPMI 1640 medium containing 5% foetal calf serum and antibiotics. All dilutions were performed at least in quadruplicate. Additional wells containing 100µl of
25   the above medium for reagent and cells only controls were also included.

TF1 cells were washed twice in serum free RPMI 1640 medium and resuspended in RPMI 1640 medium

supplemented with 5% foetal calf serum, 100U/ml

penicillin and 100μg/ml streptomycin and 4ng/ml rhIL-5

at a density of 2.5×10^5/ml. Aliquots of 100μl were

added to the previously prepared dilution series and

5   the plate incubated for 48hr. in a humidified

incubator containing 5% CO_2 at 37°C.

Cell proliferation was measured colourimetrically

by addition of 40μl CellTiter96 substrate (Promega),

returning the plate to the incubator for a further 4hr

10   and finally determining the absorbance at 490nm. The

percentage inhibition for each concentration of TGF β_2

as compared to cell only wells was then calculated.

Assay for Neutralisation of TGF β_2 Inhibitory Activity

15   by Anti-TGF β_2 Antibodies

Neutralisation of TGF β_2 was determined by making

doubling dilutions in of each purified antibody in

100μl of medium as above. TGF β_2 was added to each

antibody dilution to give a final concentration

20   equivalent to that which gave 50% inhibition in the

titration described above. Each dilution was prepared

in quadruplicate. Additional wells were prepared for

antibody only, cells only and reagent controls. Cell

preparation and determination of cell proliferation

25   was performed as described above.

Results

TGF β_2 was shown to inhibit the proliferation of

TF1 cells by 50% at a concentration of 50pM. This concentration was used for all neutralisation experiments.

5 These assays showed that TGF β_2 activity was neutralised in a dose dependant manner for both scFv fragments (figure 10) and for whole IgG4 antibodies (figure 11). The concentration of antibody which gave 50% inhibition was determined from the graphs and is shown in table 4.

10

EXAMPLE 4

Inhibition by Antibodies of TGFβ Binding to Receptors Measured in A Radioreceptor Assay

Single chain Fv fragments and whole IgG4
15 antibodies from different clones were expressed and purified and their ability to inhibit binding of TGFβ to receptors measured in a radioreceptor assay.

Purification of scFv

20 ScFvs containing a poly histidine tail are purified by immobilised metal affinity chromatography. The bacterial clone containing the appropriate plasmid is inoculated into 50 ml 2TY medium containing 2% glucose and 100 µg/ml ampicillin (2TYAG) and grown
25 overnight at 30°C. The next day the culture is added to 500 ml prewarmed 2TYAG and grown at 30°C for 1 h. The cells are collected by centrifugation and added to 500 ml 2TY containing ampicillin and 1 mM IPTG and

grown at 30°C for 4 h. The cells are then collected

by centrifugation and are resuspended in 30 ml

ice-cold 50 mM Tris HCl pH 8.0, 20% (w/v) sucrose, 1

mM EDTA. After 15 min end-to-end mixing at 4°C the

5   mixture is centrifuged at 12 k rpm for 15 min at 4°C.

The supernatant is removed and to it added ~ 1ml

NTA-agarose (Qiagen 30210) and mixed at 4°C for 30

min.   The agarose beads are washed extensively with

50 mM sodium phosphate, 300 mM NaCl and loaded into a

10   small column. After further washing with 50 mM sodium

phosphate, 300 mM NaCl, 10 mM imidazole pH 7.4 scFv is

eluted with 50 mM sodium phosphate, 300 mM NaCl, 250

mM imidazole pH 7.4. 0.5 ml fractions are collected

and the protein containing fractions identified by

15   measuring the A_{280nm}. Pooled fractions are

concentrated and scFv further purified by gel

filtration in PBS on a Superdex 75 column (Pharmacia).

Purification of Whole Antibodies

20   Whole IgG4 antibodies were purified as described

in Example 2.

Radioreceptor Assay for TGF-β

Neutralisation of TGF-β activity is measured by

25   the ability of the scFvs and IgGs to inhibit the

binding of ^{125}-I labelled TGF-β to its receptors on

A549 human lung carcinoma cells.

A549 cells (ATCC CCL 185) are grown in high

glucose Dulbecco's modified Eagle's medium (Sigma D-6546) supplemented with 10% foetal calf serum (PAA), 2 mM glutamine (Sigma G-7513), penicillin/streptomycin (Sigma P-0781), MEM non-essential amino acids (Sigma

5   M-7145).

Cells are seeded at 1-2 x 105 cells / ml / well into the wells of 24-well cluster plates and grown for 24 h in serum-free DMEM. Cell monlayers are washed twice with serum-free DMEM and 0.5 ml binding

10   medium (DMEM/Hams F12 (Sigma D-6421) containing 0.1% (v/v) BSA added to each well.

Aliqouts of ^{125}I-TGF-β1 or -β2 (70-90 TBq/mmol; Amersham International) at 20 pM are preincubated with antibody in binding medium at room temperature for 1

15   h. Duplicate samples of 0.5 ml of TGF-β/antibody mixtures are then added to the cell monlayers and are incubated at 37°C for 1-2 h. Control wells contain TGF-β only. Unbound TGF-β is removed by washing 4 times with Hank's balanced salt solution containing

20   0.1% BSA. Cells are solubilised in 0.8 ml 25 mM Tris HCl pH 7.5, 10 % glycerol, 1 % Triton X-100 at room temperature for 20 min. The contents of each well are removed and ^{125}I measured in a gamma counter. The potency of each scFv or IgG is measured by the

25   concentration of antibody combining sites necessary to inhibit binding of TGF-β by 50% (IC50; Table 5). Thus the IC50 values are below 10nM and in some cases below 1nM indicating very potent antibodies.

EXAMPLE 5

Prevention of Scar Formation by Antibodies Against TGF β1 and TGF β2 in the Injured Central Nervous System of the Rat

5   Logan *et al* (1994) Eur.3 Neuroscience 6,355-363 showed in a rat model of CNS injury, the ameliorating effect of a neutralising turkey antiserum directed against TGF β_1 on the deposition of fibrous scar tissue and the formation of a limiting glial

10   membrane that borders the lesion. A study was set up to investigate the effects of neutralising engineered human antibodies directed against both TGF β_1 and TGF β_2 in the same rat model. The derivation of the antibodies used in this study is described in examples

15   1 and 2.

Method

Animals and surgery

20   Groups of five female Sprague-Dawley rats (250g) were anaesthetised with an i.p. injection. The anaesthetised rats had a stereotactically defined lesion made into the right occipital cortex (Logan *et al* 1992 Brain Res. 587, P216-227) and the lateral

25   ventricle was surgically cannulated and exteriorised at the same time (Logan *et al* 1994 supra).

Neutralisation of TGF β

Animals were intraventricularly injected daily
with 5ul of purified anti TGF β antibodies (Table 3)
diluted in a vehicle of artificial cerebrospinal fluid
as described by Logan *et al* 1994 supra. Fourteen days

5   post lesion all animals were perfusion fixed and 7mm
polyester wax sections were processed for
histochemical evaluation of the lesion site by
immunofluorescent staining.

10   Fluorescent immunohistochemistry and image analysis
Morphological changes within the wound site were
followed by immunofluorescent staining with antibodies
to fibronectin and laminin detected with anti-species
FITC conjugates (Logan *et al* 1994 supra). These

15   changes were semi-quantitatively assessed by image
analysis using a Leitz confocal microscope linked to a
Biorad MRC500 laser scanning system. Readings were
taken at standard positions mid-way along the lesion.

20   *Results*

Effects of antibodies to TGF β at the site of CNS
injury
Quantitation of the specific relative

25   fluorescence for each of the antibodies is shown in
figure 12 a and b. Laminin is a measure of the
formation of the glial limitans externa along the
boundaries of the wound and together with fibronectin

forms a matrix of fibrous tissue within the centre of the wound. Quantitation by image analysis of these two proteins allows the degree of scarring at the wound site to be determined.

5   Compared with the saline control (fig.12 a,b), There is a considerable decrease in fibronectin and laminin immuno-localisation in the wound in the anti-TGF β antibody treated brains. Thus this indicates that these engineered human antibodies
10   directed against epitopes on TGF β_1 & TGF β_2 ameliorate the effects of injury to the CNS both separately and together. by preventing the deposition of the cellular matrix proteins fibronectin and laminin within the wound site. Previously Logan *et al* (1994
15   supra) had shown the effectiveness of a polyclonal turkey anti-sera directed against TGF β_1. This is the first report of any antibodies directed against TGF β_2 having been shown to be effective in this model.

20   *EXAMPLE 6*

Determination of Binding of 6B1 IgG4 to Active or Latent Form of TGF β_2

TGFβ_2 is synthesised and secreted exclusively as a biologically inactive or latent complex (Pircher *et*
25   *al*, (1986) Biochem. Biophys Res. Commun. <u>158</u>, 30-37). The latent complex consists of TGFβ_2 disulphide linked homodimer non-covalently associated with latency-associated peptide (LAP). Activation of TGFβ_2 occurs

when it is released from it processed precursor.
Active TGFβ$_2$ is capable of reversibly dissociating and
reassociating with the LAP, which results in the
turning on and off of its bio-activity respectively.

5   Cultured PC-3 adenocarcinoma cells (Ikeda *et al*
(1987) Biochemistry 26, 2406-2410) have been shown to
secrete almost exclusively latent TGFβ$_2$ providing a
convenient source for determination of binding to the
active or latent form of TGFβ$_2$ by the antibody 6B1

10   IgG4.

Method

Cell Culture

15   PC-3 prostatic adenocarcinoma cells were grown to
confluence in supplemented with 10% FBS. The cells
were washed 3x with PBS and cells cultured for a
further 7 days in serum free Hams F12/DMEM
supplemented with 1.4 x 10^{-5}M tamoxifen (Brown *et al*,

20   (1990) Growth Factors 3, 35-43). The medium was
removed, clarified by centrifugation and divided into
two 15ml aliquots. One aliquot was acidified for 15
min with 5M HCl by adding dropwise until the pH = 3.5
and then neutralised by the similar addition of 5M

25   NaOH/1M HEPES pH7.4. This procedure activates the
latent TGFβ2 quantitatively.

Competition ELISA

Sixteen wells of an ELISA plate were coated overnight with 100µl 200ng/ml TGFβ$_2$ in PBS at 4°C. The plate was washed 3x with PBS tween and blocked at 37°C with 200µl of 3% Marvel in PBS.

5   The following samples were incubated at room temperature for 1 hour.

400µl Hams F12/DMEM (reagent blank)

400µl Hams F12/DMEM plus 4µg 6B1 IgG4 antibody (positive control)

10   400µl PC 3 acid activated conditioned media plus 4µg 6B1 IgG4 antibody (active TGFβ$_2$ sample)

400µl PC 3 untreated conditioned media plus 4µg 6B1 IgG4 antibody (latent TGFβ$_2$ sample)

The ELISA plate was emptied of blocking solution 15   and 100µl of one of the above solutions added to sensitised wells in quadruplicate and incubated at room temperature for 2 hours. The plate was washed 3x with PBS/Tween and wells refilled with 100µl of goat anti-human IgG γ chain alkaline phosphatase conjugate 20   diluted 1:5000 in 1% Marvel/PBS. After 1 hour the wells were washed 3x with PBS/Tween and bound antibody was revealed with p-NPP substrate by absorbance at 405 nm.

25   *Results*

The results of this experiment are shown in Figure 23.

This result clearly shows that pre-incubation

with activated TGFβ2 inhibits binding of 6B1 to TGFβ2

bound onto an ELISA plate, whereas the latent form

does not. This proves that 6B1 IgG4 only binds to the

active form of TGFβ2.

5

EXAMPLE 7

Neutralisation by antibodies directed against TGFβ2 of
the inhibitory effect of TGFβ isoforms on cell
proliferation

10   The neutralising activity of 6B1 IgG4, 6H1 IgG4

(purified as in example 2) and a mouse monoclonal

antibody (Genzyme; J.R. Dasch et al., supra) was

measured for each of the TGFβ isoforms, TGFβ1, TGFβ2

and TGFβ3 in the TF1 cell proliferation assay

15   described in Example 3. The concentration of TGFβ

isoform was 100pM in each assay.

     As shown in Figure 16, 6B1 IgG4 strongly

neutralises TGFβ2 with an IC_{50} of approximately 2nM

(Table 6). This compares to 10nM for the mouse

20   monoclonal from Genzyme and 12nM for 6H1 IgG4.

Neither 6B1 IgG4 nor 6H1 IgG4 significantly neutralise

TGFβ1 (Fig. 17). However, there is significant

neutralisation of TGFβ3 by both 6B1 (IC_{50} ca. 11nM) and

6H1 IgG4 ca. 20nM; Fig. 18). This is considerably

25   less than the neutralisation potency of the Genzyme

monoclonal (IC_{50} ca. 0.1nM).

     Both 6B1 IgG4 and 6H1 IgG4 are stronger

neutralisers of TGFβ2 activity than of TGFgβ3

activity. The neutralisation of TGFβ3 activity is greater than would be predicted from the relative binding of these two isoforms by the antibodies (example 2) and the relative binding in a

5   radioreceptor assay (example 8).

EXAMPLE 8

Inhibition by antibodies directed against TGFβ2 of binding of other TGFβ isoforms to receptors measured

10 *in a radioreceptor assay*

The ability of 6B1 IgG4 to inhibit binding of TGFβ isoforms to receptors was measured in a radioreceptor assay as described in example 4.

6B1 IgG4 inhibited binding of ^{125}I-TGFβ2 with an

15 IC_{50} of 0.05nM. There was no significant inhibition of binding of ^{125}I-TGFβ1 whereas for ^{125}I-TGFβ3 6B1 IgG4 inhibited binding with an IC_{50} of approximately 4nM (Table 6). This indicates the potency of 6B1 IgG4 in this assay and its selectivity for the neutralisation

20 of TGFβ2 activity. Cross-reactivity with TGFβ3 in this assay is less than 2%.

Thus 6B1 IgG4 preferentially inhibits the binding of TGFβ2 to its receptors compared with binding of TGFβ3.

25

EXAMPLE 9

Assessment of TGFβ1 Antibodies for Therapeutic Use

The antibodies isolated in Example 1 were

assessed for potential therapeutic value by *in vitro* measurements of the ability to inhibit TGFβ1 binding to its receptors and *in vitro* binding properties.

5 In Example 4 (Table 5) CS32 showed the strongest inhibition of the antibodies tested of the binding of ^{125}I-TGFβ1 to receptors on A549 cells. A further comparison was performed between CS32 and further antibodies (CS35, CS37 and CS38) that were isolated as described in the experiment in Example 1, section 5c.

10 This showed that CS37 appeared to be the most potent of these antibodies in this assay with an IC$_{50}$ of approximately 8nM, compared with 40nM for CS32. The IC50 value for CS32 is higher than in the previous assay (Table 5) because the nature of the assay means

15 that the absolute IC$_{50}$ value can vary with assay conditions.

The antibodies 1A-E5 and 1AH-6 (Example1, section 1f) and antibodies derived from them were much less potent than antibodies derived from 1B2 in

20 neutralising TGFβ activity in this radioreceptor assay.

Thus CS37 was the most potent antibody candidate as assessed by inhibition of binding of ^{125}I-TGFβ1 to its receptor.

25

Assessment of binding to TGFβ3 by anti-TGFβ1 antibodies

The antibodies 14A1 and 10A6 (Example 1, section

2 (a) (iii)) were shown to preferentially bind TGFβ1 over TGFβ2 and TGFβ3 using the same specificity ELISA as was described in Example 1, section 1 (d) (iii), except that microtitre plates were coated with 50μl of

5 either 0.2μg/ml TGFβ1; 0.2μg/ml TGFβ2; 0.2 μg/ml TGFβ3; 10μg/ml bovine serum albumin (BSA) or PBS (the uncoated well). The clones were shown to be specific for TGFβ1 since the signal generated in the TGFβ1 coated well was at least five fold greater than the

10 signal on TGFβ2 and TGFβ3.

Antibodies derived from the same 1B2 lineage as these antibodies, such as 27C1/10A6 IgG4 (which contains the same VL as 10A6 and the 27C1 VH was prepared by mutagenesis of CDR3 residues) should have

15 the same cross-reactivity against TGFβ3.

EXAMPLE 10

Construction of a High Expressing Cell Line for 6B1 IgG4 using the Glutamine Synthase Selection Systemand

20 *Assessment in a Neutralisation Assay*

Construction of p6H1 VH gamma4

6B1 VH was amplified from 6H1 pG4D100 (Example 2) by PCR using oligonucleotides P16 and P17. This DNA

25 was joined by PCR with a 158bp DNA fragment from M13VHPCR1 (R. Orlandi et al Proc. Natl. Acad. Sci. USA 86 3833-3837, 1989) containing a signal sequence, splice sites and an intron, using oligonucleotides P10

and P17. The PCR product was cut with HindIII ad ApaI and cloned into HindIII-ApaI cut pGamma4 (Lonza Biologics plc). A plasmid with the correct insertion was identified and designated p6H1 VH gamma4 (see
5   Figure 20). The VH gene and flanking regions were sequenced at this stage.

Construction of 6B1ΔBam pLN10

     The VL gene of 6B1 was amplified from the clone
10   of 6B1 scFv in pCANTAB6 (Example 1) and subcloned into pUC119. The VL gene was then mutated by in vitro mutagenesis to remove an internal BamHI site, modifying the DNA sequence but not the protein sequence. In vitro mutagenesis was performed using
15   the oligonucleotide LamDeltaBamHI (Table 1) using a kit from Amersham International plc. The mutated VL gene was amplified using the primers Vλ3backEuApa and HuJλ2-3ForEuBam and subcloned as an ApaLI-BamHI fragment into the vector vlcassetteCAT1. The VL gene
20   was then cloned as a HindIII-BamHI fragment into the vector pLN10 (Figure 8) to generate the vector 6B1ΔBam pLN10.

Construction of p6B1λ

25   The 6B1 Vλ gene was amplified by PCR from p6B1ΔBampLN10 using oligonucleotides P22 and P26. The Cλ gene was amplified by PCR from pLN10-10A6 (Example 2) using oligonucleotides P25 and P19. The 2 DNAs

were joined by overlapping PCR using the oligonucleotides P22 and P19 and the product cut with BstBI and EcoRI and cloned into BstBI-EcoRI cut pMR15.1 (Lonza Biologics plc). A plasmid with the

5   correct insertion was identified and designated p6B1λ (Figure 21).

Construction of final expression vector p6B1gamma4gs

p6H1 VHgamma4 and p6B1λ were digested with BamHI

10   and NotI, fragments were purified and ligated together. A plasmid of the desired configuration was identified from transformants and designated p6B1gamma4gs (Figure 22).

15   Transfection of NSO with p6B1 gamma4gs

Stable transfectants secreting 6B1 IgG4 were selected by introducing into NSO myeloma cells p6B1 which includes the glutamine synthetase (gs) gene which allows growth in glutamine-free (G-) medium

20   (C.R. Bebbington et al Bio/Technology $\underline{10}$ 169-175, 1992). 40µg p6B1 gamma4gs were linearised by digestion with PvuI. The DNA was electroporated into 1.5×10^7 NSO cells. Cells were then added to G+DMEM/10% FCS and 50µl aliquots distributed into 6 x

25   96-well plates and allowed to recover for 24h. The medium was then made selective by the addition of 150µl G-DMEM/10%FCS. Three weeks later gs$^+$ transfectants were screened by ELISA for the ability

to secrete human IgG4λ antibody. The highest producers were expanded and further analysed. From this analysis 5D8 was selected as the candidate production cell line. 5D8 was cloned once by limiting

5 dilution to give the cell line 5D8-2A6.

Assessment of 6B1 IgG4 derived from cell line 5D8-2A6 in the TF1 neutralisation assay

6B1 IgG4 was purified from the GS/NS0 cell line

10 5D8-2A6 grown in serum-free medium as described in Example 2. The 6B1 IgG4 antibody was assayed in the TF1 neutralisation assay as described in Example 3. An IC_{50} value of 1.8nM was obtained in this assay. Subsequent assays of preparations of 6B1 IgG4 derived

15 from the 5D8-2A6 cell line have indicated values of IC_{50} in the range of 0.65 to 2nM. These are comparable to the values obtained for 6B1 IgG4 produced from CHO cells (Example 2) and compare favourably with that obtained for 6H1 IgG4 derived from a CHO cell line

20 (IC_{50} of 15nM). The values obtained for the IC_{50} for 6B1 IgG4 and 6H1 IgG4 in this example are more reliable than those obtained in Example 3 and are shown in Table 4, because of improvements in the assay and in the expression and purification of the

25 antibodies. The IC_{50} value may however be expected to vary with the precise conditions of the assay.

Thus the 6B1 IgG4 provides potent neutralisation of TGFβ2 and is suitable for use as a therapeutic.

EXAMPLE 11

*Determination of the Epitope on TGFβ2 for the Antibody
6B1 using a Peptide Phage Display Library*

5   The antibody 6B1 was further characterised by
epitope mapping. This was done by using a peptide
phage display library to select peptide sequences that
bind specifically to 6B1. These peptide sequences
were then compared to the amino acid sequence of

10   TGFβ2. Correlation between peptide sequences that
bind to 6B1 and matching parts of the TGFβ2 amino acid
sequence indicate an epitope of TGFβ2 to which 6B1
binds. An "epitope" is that part of the surface of an
antigen to which a specific antibody binds.

15   In this example, the peptide library used was
constructed as described by Fisch et al (I. Fisch et
al (1996) Proc. Natl. Acad. Sci USA 93 7761-7766) to
give a phage display library of 1×10^{13} independent
clones. Phage displaying peptides that bind to the

20   antibody 6B1 were selected from this library by
panning. This was performed as described in Example
1.

      Purified 6B1 IgG4 antibody at 10μg/ml in 4ml of
PBS was coated onto a plastic tube (Nunc; maxisorp) by

25   incubating overnight at 4°C. After washing and
blocking with MPBS (see Example 1) an aliquot of the
peptide library containing 5×10^{13} phage in 4ml 3%MPBS
was added to the tube and incubated at room

temperature for 1.5 hours. The tube was washed 10
times with PBST(0.1%), then 10 times with PBS. Bound
phage particles were eluted from the tube by adding
4ml of 100mM triethylamine and incubating the tube
5   stationary for 10 minutes at room temperature. The
eluted phage were then added to a tube containing 2ml
1M-Tris.HCl (pH7.4) and 10ml 2YT broth. The phage
were then added to 20ml of logarithmically growing E.
coli TG1 cells and grown for 1 hour shaking at 100rpm
10   at 37°C. The infected cells were then plated on 2YT
agar medium with 15µg/ml tetracycline in 243mm x 243mm
dishes (Nunc). Plates were incubated at 30°C for 18
hours. Colonies were scraped off the plates into 10
ml 2TY broth containing 15% (v/v) glycerol for storage
15   at -70°C.

250µl of cells from the first round of selection
was used to inoculate 500ml 2YT broth (containing
15µg/ml tetracycline) in a 2 litre conical flask and
grown overnight, at 30°C with shaking at 280rpm. A
20   2ml aliquot of this culture was then taken and
centrifuged to remove all cells. 1ml of this phage
supernatant was the used for a second round of
selection as described above. The pattern of phage
growth and panning was repeated over a third and a
25   fourth round of selection.

Individual colonies from the fourth round of
selection were used to inoculate 100µl 2YT broth
(containing 15µg/ml tetracycline) into individual

wells of 96 well tissue culture plates and grown overnight with gentle shaking at 100rpm at 30°C. Glycerol was added to a final concentration of 15% (v/v) and these master plates were stored frozen at

5 -70°C.

These clones were screened for clones that bound specifically to the antibody 6B1 in ELISA. Cells from the master plates were used to inoculate 96 well tissue culture plates containing 100µl 2YT broth

10 (containing 15µg/ml tetracycline) per well and grown overnight with gentle shaking at 100rpm at 30°C. The plates were then centrifuged at 2000rpm. The 100µl phage supernatants from each well were recovered and each was mixed with 100µl of 4% skimmed milk powder in

15 2x PBS. 100µl of each of these was then assayed by phage ELISA. Purified 6B1 IgG4 antibody at 10µg/ml in PBS was coated onto flexible microtitre plates by incubating overnight at 4°C. Control plates coated with an irrelevant IgG4 antibody at 10µg/ml were also

20 prepared. The ELISAs were performed as described in Example 1, and visualised with the chromagenic substrate pNPP (Sigma).

Approximately 20% of all the clones analysed bound to the 6B1 coated plate. None of the clones

25 analysed bound to ELISA plates coated with the irrelevant antibody. Binding therefore appeared to be specific for the binding site of the antibody 6B1.

Clones which bound 6B1 were analysed by DNA

sequencing as described by Fisch et al. A total of 31
different clones were sequenced. These were analysed
for possible matches with the sequence of TGFβ2 using
Mac vector software. Of these clones, 12 showed poor

5   matching with the sequence of TGFβ2 and 10 had no
similarity at all. However, there were 4 different
clones (some of which had been selected more than
once) which showed a reasonable match to a region of
the TGFβ2 sequence between amino acid positions 56 to

10   69. Table 8 shows the amino acid sequence of the exon
of each of these clones that appears to be responsible
for binding to 6B1.

None of these clones exactly match the sequence
of TGFβ2 nor is there a single clear consensus

15   sequence between the peptide clones. Nevertheless,
careful examination of the sequences reveals a match
with residues 60 to 64 of TGFβ2 (Table 8). Lining up
four clones with L at position 64 reveals 2 clones
with R at position 60, 1 clone with V at position 61,

20   2 with L at position 62 and 3 with S at position 63.
This provides the sequence RVLSL corresponding to
residues 60 to 64 which form part of the alpha helix
which forms the heel region of TGFβ2. An antibody
recognising this structure would not be expected to

25   make contact with every amino acid residue in the
helix and so a peptide mimicking this sequence could
have considerable sequence variation at positions that
correspond to parts of the helix that do not make

contact. The alpha helix recognised is believed to form part of the receptor binding region of TGFβ2 (D.L. Griffith et al.(1996) Proc. Natl. Acad. Sci. USA 93 878-883).

EXAMPLE 12

Determination by Immunohistochemistry of Binding of 6B1 IgG4 to TGFβ2 in Mammalian Tissue and Absence of Cross Reactivity

To detect TGFβ2 in formalin-fixed tissue sections that express the cytokine, the tissue section is generally treated with a protease, pronase E. This digestion step unmasks the antigen, possibly activating latent TGF β2 to give active TGF β2. 6B1 IgG4 detects only the active form of TGF β2 (Example 6).

Using 6B1 IgG4 and immunohistochemical methods the distribution of TGF β2 was determined in formalin fixed-paraffin wax embedded rat normal rat kidney, and experimentally lesioned rat brain tissue, following pronase E digestion.

The reactivity of 6B1 IgG4 in frozen cryostat sections of acetone post-fixed normal human tissue was also ascertained to determine whether there was any binding to other antigens in these tissues.

Method

Rat Tissue

Paraffin embedded rat tissues were de-waxed and
rehydrated through an alcohol series. The sections
were then treated with 0.1% pronase E for exactly 8
5   min and then washed in water. TGF β2 was detected in
the sections using 6B1 IgG4 at 500ng/ml following the
protocol provided with a Vectastain ABC (avidin-
biotin-complex) kit from Vector Laboratories. On
kidney sections, bound antibody was located with
10   alkaline phosphatase and peroxidase was used on rat
brain tissues.

Human Tissue

The following human tissue samples were used:
15   Adrenal, Aorta, Blood, Large intestine, Small
intestine, Cerebrum, Kidney, Lymph Node, Liver, Lung,
Spleen, Pancreas, Skeletal muscle, Cardiac Muscle,
Thyroid, Nerve, Skin, Eye.

Cryostat sections and smears were fixed for 15
20   minutes in acetone before application of 6B1 IgG4
antibody labelled with FITC using Sigma Immunoprobe
kit. The labelled antibody was incubated for 18hr at
4°C, then detected using an indirect alkaline
phosphatase method (detection with anti-FITC antibody
25   followed with anti-species enzyme conjugated
antibody). In instances where endogenous alkaline
phosphatase activity could not be suppressed a
peroxidase detection method was used. No pronase

digestion was used in this case, therefore this procedure would detect only antigens with which the antibody cross-reacts.

5   *Results*

Rat Tissue

Rat kidneys displayed positive staining in tubules present on both the apical and the basolateral
10   side, demonstrating the presence of TGF β2 in the tissues.

Injured rat brain at 5 days post injury showed positive staining of neurones, astrocytes and macrophages which was absent in normal brain. This
15   indicates that the TGF β2 is expressed in rat brain following injury.

Human Tissue

No specific staining of any tissue was observed
20   using fixed cryostat sections of the tissues listed above. Therefore 6B1 IgG4 does not cross-react with antigens in these tissues and when used therapeutically will bind only active TGF β2 in tissue sections detected by immunohistochemical methods.

25

EXAMPLE 13

Kinetic analysis of the binding of 6B1 single chain Fv and 6B1 IgG4 to TGFβ isoforms

Surface plasmon resonance (SPR) can be used to examine real-time interactions between an immobilised ligand and an analyte, and derive kinetic constants from this data. This was performed using the BIAcore
5   2000 system (Pharmacia Biosensor) with the antigen immobilised on a surface, and the antibody as analyte.

The system utilises the optical properties of surface plasmon resonance to detect alterations in protein concentration within a dextran matrix.
10 Antigen is covalently bound to the dextran matrix at a set amount, and as solution containing antibody passes over the surface to which this is attached, antibody binds to the antigen, and there is a detectable change in the local protein concentration, and therefore an
15 increase in the SPR signal. When the surface is washed with buffer, antibody dissociates from the antigen and there is then a reduction in the SPR signal, so the rate of association, and dissociation, and the amount of antibody bound to the antigen at a
20 given time can all be measured. The changes in SPR signal are recorded as resonance units (RU), and are displayed with respect to time along the y-axis of a sensorgram.

The density of immobilised ligand on the surface
25 of a BIACore chip is important when deriving kinetic data from the sensorgrams generated. It needs to be quite low, so that only a small amount of analyte antibody is needed for saturation of the chip surface.

For simplicity, the density of a chip surface is quoted in RU's, and an ideal amount for a ligand such as TGFβ2 or TGFβ3 (25kDa) is 400-600 RU's relative to the baseline set during the immobilisation of the

5   ligand to the surface. The actual amount of TGFβ that has to be added to get the correct density has to be determined by investigation, but is reproducible once the correct concentration has been found.

Immobilisation of the ligand to the dextran

10   matrix of the chip surface is facilitated via amine groups, on lysine side chains in the protein, and carboxyl groups in the dextran matrix. The carboxyl groups in the dextran are activated with N-hydroxysuccinimide (NHS) and N-ethyl-N'-(3-

15   diethylaminopropyl) carbodiimide (EDC) the antigen in acidic solution is then bound to the surface, and finally any unreacted carboxyl groups are blocked with ethanolamine.

The immobilisation of ligand is automated by the

20   BIACore 2000 machine, and all steps are carried out in the autosampler or in the flowcell, on the dextran surface of the chip. The buffer used throughout the immobilisation procedure, and the analysis of samples is Hepes -buffered saline (HBS) with a surfactant

25   (Pharmacia Biosensor). The chips (Pharmacia, CM5), have dextran coating on a thin layer of gold. NHS at 100mM and EDC at 400mM are mixed by the autosampler, and then a fixed volume is injected over the flowcell

surface. This is followed by an injection of antigen in a suitable buffer. In the case of TGFβ, a surface of the correct density was given by using 25-30μg/ml solution of TGFβ2 (AMS) OR TGFβ3 (R & D systems) in 10mM acetate. After injection of the ligand, the chip is blocked using 1M ethanolamine. The total amount of TGFβ bound was assessed from the total increase in resonance units over this period.

To determine the kinetic parameters, a series of dilutions of the antibody samples was made in HBS from about 500μg/ml down to less than 1 μg/ml, usually through doubling dilutions. After the antibody has been injected over the antigen surface, the surface is washed with HBS, then regenerated by stripping off the bound antibody with a pulse of 100mM HCl. At the higher concentrations of antibody the antigen on the chip surface is saturated, and the off rate is determined on washing with buffer in the dissociation phase. For determination of the on-rate, lower concentrations of antibody are used, giving a linear binding phase in the sensorgram, allowing k_{on} determination.

The set of dilutions were repeated on a separate preparation of the same antibody.

To manipulate the sensorgrams to obtain kinetic constants k_{on} and k_{off}, the BIAevaluation software package is used. For each binding curve used in the calculations, care was taken that the conditions were

appropriate for the determination of kinetic
constants.

6B1 IgG4 was purified from the GS/NS0 cell line
of Example 10 as in Example 2. 6B1 single chain Fv
5   was expressed intracellularly in *E. coli*, refolded *in
vitro* (using the methodology of WO94/18227), and
purified to give a homogeneous product. The values of
k_{on} and k_{off} were determined for 6B1 IgG4 for binding
to both TGFβ2 and TGFβ3, and for the single-chain Fv
10   6B1 for binding to TGFβ2. The dissociation constant
was calculated by dividing k_{off} by k_{on}. The values for
these kinetic parameters are shown in Table 7.

Thus, 6B1 scFv and 6B1 IgG4 show very low
dissociation constants of 2.3nM and 0.89nM
15   respectively for TGFβ2, and there is 9% cross-
reactivity with TGFβ3 (as judged by the ratio of
dissociation constants of 6B1 IgG4 for TGFβ3 and
TGFβ2). For comparison, in earlier studies, where the
standard errors were greater and the values less
20   precise, the Kd values for TGFβ2 were determined to be
0.7nM for 6A5 scFv (Table 2) and 2nM for 6H1 IgG4
(Example 2). The Kd values for all the antibodies
directed against TGFβ2 which share the same 6H1 VH
domain are low and below 10nM.

25

EXAMPLE 14

*Binding of a Peptide Corresponding to Residues 56 to
⌐f TGFβ2 to 6B1 IgG4*

A peptide was synthesised corresponding to the amino acids of TGFβ2 surrounding the residues RVLSL, the epitope identified from the selection of phage from the peptide display library (Example 11).

5   The 17-mer peptide CGG-TQHSRVLSLYNTIN (TGFβ2$_{56-69}$; synthesised by Cambridge Research Biochemicals) contains residues 56 to 69 of TGFβ2 with RVLSL (residues 60 to 64) at its centre. The CGG N-terminal extension is a spacer with a cysteine residue to

10   facilitate coupling of the peptide to carrier proteins. The peptide corresponding to residues 56 to 69 from TGFβ1 (TGFβ1$_{56-69}$; CGG-TQYSKVLSLYNQHN) was also synthesised. As a control, irrelevant peptide GPEASRPPKLHPG was used.

15   Two approaches were used to confirm that the epitope on TGFβ2 for 6B1 IgG4 comprised the amino acids RVLSL.

(i) Assessment of the ability of 6B1 IgG4 to bind to TGFβ2$_{56-69}$ and TGFβ1$_{56-69}$ coupled to BSA by ELISA

20   (ii) Assessment of the ability of peptides to bind to 6B1 IgG4 coated onto a BIACore sensor chip.

(i) Assessment of the ability of 6B1 IgG4 to bind to TGFβ2$_{56-69}$ and TGFβ1$_{56-69}$ coupled to BSA by ELISA

25   The binding of 6B1 IgG4 to synthetic peptides TGFβ1$_{56-69}$ and TGFβ2$_{56-69}$ conjugated to BSA was assessed in an ELISA assay. This was compared with the binding of a control antibody 2G6 IgG4 which is an engineered

antibody with a heavy chain containing a VH from an antibody directed against the hapten NIP combined with a light chain containing a VL from an antibody directed against lysozyme.

5

Method

Two mg of each of the peptides TGF$\beta 1_{56-69}$ and TGF$\beta 2_{56-69}$ were conjugated to BSA using an Imject Activated Immunogen Conjugation kit (Pierce).

10 An immunosorp microtitre plate (Nunc) was coated overnight with 10ug/ml of the conjugated peptides in PBS (rows A-D TGF$\beta 1_{56-69}$, rows E-F TGF$\beta 2_{56-69}$) at 100μl/well. The wells were washed 3x with PBS-tween and the following additions made: Column 1 -100μl PBS
15 in each well as reagent control; Column 2, rows A,B,E and F 200μl of 6B1 IgG4 10μg/ml; Column 2, rows C,D,G and H 200μl of 2G6 IgG4 10μg/ml.

100μl of PBS was put into all the remaining wells. To produce doubling dilutions of the
20 antibodies, 100μl was removed from each well in column 2 and placed into the next well in column 3. The sample was mixed and 100μl removed and added to the next well in column 4. This procedure was repeated along the plate with the last 100μl being discarded.
25 The plate was then incubated at 4°C for 18hr.

After 3x washes with PBS-tween the wells were refilled with 100ul of an alkaline phosphatase conjugate of goat F(ab')$_2$ fragment specific for the

human IgG gamma chain diluted 1:1000 in PBS and incubated for a further 1hr. After 3x further washes with PBS-tween bound antibody was revealed with p-NPP substrate for 20min.

5

Results

6B1 IgG4 was shown to bind to both conjugated peptides (Figure 15) but the ELISA signal obtained with $TGF\beta1_{56-69}$ was much lower than that obtained with

10 $TGF\beta2_{56-69}$ at an equivalent concentration of 6B1 IgG4. An approximately 8 to 10 times higher concentration of 6B1 IgG4 was required to obtain an equivalent signal with $TGF\beta1_{56-69}$ compared with $TGF\beta2_{56-69}$. No signal was obtained with the control 2G6 IgG4 antibody with

15 either peptide-BSA conjugate. 6B1 IgG4 therefore strongly binds TGFb256-69 and more weakly binds $TGF\beta1_{56-69}$ coupled to BSA.

(ii) Assessment of the ability of peptides to bind to

20 *6B1 IgG4 coated onto a BIACore sensor chip.*

The binding of 6B1 IgG4 to $TGF\beta2_{56-69}$ was confirmed by binding the peptide to 6B1 IgG4 coated on to a BIACore sensor chip. The determination of binding properties by surface plasmon resonance using

25 the Pharmacia BIACore 2000 was described in Example 13. The method of creating a BIACore sensor chip coated with 6B1 IgG4 was as for the method for coupling with $TGF\beta$, described in Example 13, except

that 6B1 IgG4 was coupled at 5μg/ml in 10mM acetate buffer, pH3.5. A surface of 5000RU was generated using 25μl of 6B1 IgG4.

5   Twenty μl of the the peptides were applied to the 6B1 surface at 1mg/ml with regeneration of the surface using an acid pulse to remove bound peptide between samples. The amount of binding was assessed by setting a baseline response of absolute RU prior to injection, and then subtracting this from the value at 10   20 seconds after the injection was complete to give a relative response in RU. This is taken to be the amount of binding to the 6B1 surface.

     The binding obtained is shown in Table 9. There was a very low level of binding of the irrelevant 15   peptide. $TGF\beta 1_{56-69}$ appeared to bind specifically at a low level to 6B1 IgG4. However, the $TGF\beta 2_{56-69}$ peptide bound to 6B1 IgG4 specifically and very much more strongly.

     The low level of binding of 6B1 IgG4 to the $TGF\beta 1$ 20   peptide in the ELISA and BIACore assays is not unexpected given that 10 of the 14 $TGF\beta$ amino acids are identical with the $TGF\beta 2$ peptide. Nevertheless, 6B1 IgG4 binds the $TGF\beta 2_{56-69}$ peptide very much more strongly than it binds the $TGF\beta 1_{56-69}$ peptide. The 25   level of discrimination between these $TGF\beta 1$ and $TGF\beta 2$ peptides is very much lower however than is seen for the radioreceptor (Table 6) and neutralisation assays (Table 6 and Figures 16 and 17) with native isoforms.

binding and neutralising the biological activity of TGFβ2.

If the epitope for 6B1 IgG4 is three dimensional there may be other non-contiguous epitopes to which
5   the antibody may bind.

There is earlier evidence that antibodies directed against this region of TGFβ2 may be specific for TGFβ2 and neutralise its activity. Flanders et al (Development <u>113</u> 183-191 1991) showed that polyclonal
10   antisera could be raised in rabbits against residues 50 to 75 of mature TGFβ2 and that these antibodies recognised TGFβ2 but not TGFβ1 in Western blots. In an earlier paper, K.C. Flanders et al (Biochemistry <u>27</u> 739-746, 1988) showed that polyclonal antisera raised
15   in rabbits against amino acids 50 to 75 of TGFβ1 could neutralise the biological activity of TGFβ1. The antibody we have isolated and characterised, 6B1 IgG4, is a human antibody directed against amino acids in this region which neutralises the biological activity
20   of human TGFβ2. It is surprising that such a neutralising antibody against TGFβ2 can be isolated in humans (where immunisation with a peptide cannot be used for ethical reasons) directly from a phage display antibody repertoire.

Table 1: Oligonucleotide primers used in the identification and characterisation of TGF-b1 antibodies.

Primer | Nucleotide sequence 5' to 3'

1B2 mutVHCDR3: 5' CGT GGT CCC TTT GCC CCA GAC GTC CAC ACC ACT AGA ATC GTA GCC ACT ATA TTC CCC AGT TCG CGC ACA GTA ATA CAC AGC CGT

pUC19reverse: 5' AGC GGA TAA CAA TTT CAC ACA GG 3'

fdtet seq: 5' GTC GTC TTT CCA GAC GTT AGT 3'

PCR-H-Link: 5' ACC GCC AGA GCC ACC TCC GCC 3'

PCR-L-Link: 5' GGC GGA GGT GGC TCT GGC GGT 3'

myc seq 10: 5' CTC TTC TGA GAT GAG TTT TTG 3'

HuJH4-5For: 5' TGA GGA GAC GGT GAC CAG GGT TCC 3'

RL1: 5' G(C/A)A CCC TGG TCA CCG TCT CCT CA GGT GGA GGC GGT TCA GGC GGA GGT GGC AGC GGC GGT GGC GGA TCG 3'

RL2: 5' GGA CAA TGG TCA CCG TCT CTT CA GGT GGA GGC GGT TCA GGC GGA GGT GGC AGC GGC GGT GGC GGA TCG 3'

RL3: 5' GGA CCA CGG TCA CCG TCT CCT CA GGT GGA GGC GGT TCA GGC GGA GGT GGC AGC GGC GGT GGC GGA TCG 3'

VH1b/7a back Sfi
5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG (AG)TG CAG CTG GTG CA(AG) TCT GG-3'

VH1c back Sfi
5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC (GC)AG GTC CAG CTG GT(AG) CAG TCT GG-3'

VH2b back Sfi
5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG (AG)TC ACC TTG AAG GAG TCT GG-3'

VH 3b back Sfi
5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC (GC)AG GTG CAG CTG GTG GAG TCT GG-3'

VH3c back Sfi
5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTG CAG CTG GTG GAG (AT)C(TC) GG-3'

VH4b back Sfi
5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTA CAG CAG TGG GG-3'

VH4c back Sfi
5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG (GC)TG CAG CTG CAG GAG TC(GC) GG-3'

VH5b back Sfi
5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GA(AG) GTG CAG CTG GTG CAG TCT GG-3'

VH 6a back Sfi
5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTA CAG CTG CAG CAG TCA GG-3'

VH3BACKSfiEu   5'- AGC TCG GTC CTC GCA ACT GCG GCC CCT GGG GCC CAC AGC GAG GTG CAG CTG GTG GAG TCT GG - 3'

VHJH6FORBam   5'-CGA GTC ATT CTG CAC TTG GAT CCA CTC ACC TGA GGA GAC GGT GAC CGT GGT CCC - 3'

DeltaBamHI   5'-GA GAA TCG GTC TGG GAT TCC TGA GGG CCG G-3'

Vλ3/4BackEuApa   5'- AGC TCG GTC CTC GCA ACT GGT GTG CAC TCC CAC GTT ATA CTG ACT CAG GAC CC -3'

HuJλ2-3ForEuBam 5'-G GTC CTC GCA ACT GCG GAT CCA CTC ACC TAG GAC GGT CAG CTT GGT CCC- 3'

VHJH1-2FORBam 5'-CGA GTC ATT CTG CAC TTG GAT CCA CTC ACC TGA GGA GAC GGT GAC CAG GGT GCC - 3'

VK2BackEuApa   5'- AGC TCG GTC CTC GCA ACT GGT GTG CAC TCC GAT GTT GTG ATG ACT CAG TCT CC-3'

HuJKForEuBam 5'-G GTC CTC GCA ACT GCG GAT CCA CTC ACG TTT GAT ATC CAC TTT GGT CCC -3'

Vλ3BackEuApa 5'- AGC TCG GTC CTC GCA ACT GGT GTG CAC TCC TCG TCT GAG CTG ACT CAG GAC CC -3'

LamDeltaBamHI 5'- C CGG CCC TCA GGA ATC CCA GAC CGA TTC TC- 3'

P10   5'-CTA AGC TTA CTG AGC ACA CAG GAC CTC ACC-3'

P16   5'-TTT GGA TAT CTC TCC ACA GGT GTC CAC TCC GAG GTG CAG CTG GTG GAG TCT G-3'

P17   5'-ATG GGC CCT TGG TGG AAG CTG AAG AGA CGG TGA CCA GGG TGC C-3'

P19   5'-TTG AAT TCA GGT GGG GGC ACT TCT CCC TCT ATG AAC ATT CCG TAG GGG CCA CTG TCT TC-3'

P22   5'-TTA ACG ATT TCG AAC GCC ACC ATG GGA TGG AGC TGT ATC ATC CTC-3'

P25   5'-GTC CTA GGT GAG TAG ATC TAT CTG GGA TAA GCA TGC TGT TTT C-3'

P26   5'-GAT CTA CTC ACC TAG GAC GGT CAG CTT GG-3'

Table 2 Properties of single chain Fv fragments for binding to TGFbeta1 or
TGFbeta2 determined using BIACore

Antibody	$k_{off} (s^{-1})$	$K_d (nM)$
TGFbeta1		
31G9	9.0×10^{-4}	12
CS32	1.2×10^{-3}	
CS39	1.7×10^{-3}	
TGFbeta2		
6A5	1.4×10^{-4}	0.7
6B1	6.0×10^{-4}	
6H1	1.1×10^{-3}	
14F12	2.1×10^{-3}	

Table 3 Daily dose levels for individual animals in each group

Group	Clone	Antibody format	Antigen	Dose
1	Saline Control	-	-	-
2	31G9	scFv	TGF β_1	20ng
3	6A5	scFv	TGF β_2	20ng
4	27C1/10A6	IgG4	TGF β_1	692ng
5	6H1	IgG4	TGF β_2	1.76μg
6	31G9 +6A5	scFv's	TGF β_1 TGF β_2	20ng "
7	27C1/10A6 + 6H1	IgG4's	TGF β_1 TGF β_2	692ng 1.76μg

Table 4 I.C.$_{50}$ values for antibodies in TF1 assay

Antibody	scFv (nM)	IgG4 (nM)
6H1	1.5	100
6B1	15	11
6A5	8	150
14F12	90	nd

nd = not determined

Table 5 IC$_{50}$ values for antibodies measured using a radioreceptor assay.

Anti-TGF-β1 antibody	IC$_{50}$, nM
7A3 scFv	>100
31G9 scFv	30
CS32 scFv	4.5
CS39 scFv	~60
27C1/10A6 IgG	9
VT37 scFv	~100

Anti-TGF-β2 antibody	IC$_{50}$, nM
6A5 scFv	1.5
6A5 IgG	~6
6B1 scFv	0.3
6B1 IgG	0.6
6H1 scFv	0.22
6H1 IgG	~10
11E6 IgG	1.6
14F12 scFv	3
VT37 scFv	2

Table 6 Potency of neutralisation of TGFbeta isoforms

TF1 cell proliferation assay IC_{50} (nM IgG)		
	6B1 IgG4	Genzyme
TGFbeta1	>100	1.5
TGFbeta2	2	10
TGFbeta3	11	0.1
A549 cell radioreceptor assay IC_{50} (nM IgG)		
	6B1 IgG4	Genzyme
TGFbeta1	>400	0.55
TGFbeta2	0.05	0.5
TGFbeta3	4	0.03

Table 7   Kinetic parameters of 6B1 IgG4 and 6B1 single chain Fv

antibody format	antigen	k_{off} s^{-1}	k_{on} $M^{-1}s^{-1}$	dissociation constant K_d nM
6B1 scFv	TGFβ2	6.68×10^{-4}	2.87×10^5	2.32
6B1 IgG4	TGFβ2	3.36×10^{-4}	3.84×10^5	0.89
6B1 IgG4	TGFβ3	4.5×10^{-4}	4.5×10^4	10.0

Table 8   Peptide sequences from phage binding to 6B1 IgG4

This table shows the amino acid sequence of 4 phage peptide
display clones that show a match with the sequence of
TGFbeta2. These clones have been lined up below the
relevant part of the sequence of TGFbeta2, which is shown
from amino acid positions 56 to 77.

```
TGFbeta2       TQHSRVLSLYNTINPEASASPC
Clone 1             RQLSLQQRMH
Clone 2          DPMDMVLKLC
Clone 3        WSEFMRQSSL
Clone 3             VESTSLQFRG
```

peptide	concentration of peptide, μM	amount of binding to 6B1 IgG4 surface, RU
TGFβ2$_{56-69}$	537	1012.8
TGFβ1$_{56-69}$	524	190.7
irrelevant peptide	745	60.9

Table 9 Binding of peptides from TGFbeta to 6B1 IgG4 immobilised on a
BIACore chip

CLAIMS:

1.   A specific binding member comprising a human antibody antigen binding domain specific for human TGFβ which binds the human TGFβ isoforms TGFβ2, TGFβ1, or

5   TGFβ2 and TGFβ1, preferentially over TGFβ3.

2.   A specific binding member according to claim 1 which neutralises TGFβ2, TGFβ1, or TGFβ2 and TGFβ1.

3.   A specific binding member according to claim 1 or claim 2 wherein said human antibody antigen binding

10   domain is for the TGF-β isoform TGF-β2.

4.   A specific binding member according to claim 3 wherein said human antibody antigen binding domain comprises a VH domain which has the amino acid sequence shown in Figure 2(a) (i) or Figure 2(a) (ii).

15   5.   ·A specific binding member according to claim 3 or claim 4 wherein said human antibody antigen binding domain comprises a VL domain which has the amino acid sequence shown in any of Figures 2(b) (i) to (v)

6.   A specific binding member according to claim 5

20   wherein said human antibody antigen binding domain comprises a pairing of a VH domain and a VL domain selected from:

(a)   6H1 VH, of which the amino acid sequence is shown

in Figure 2(a) (i), and 6B1 VL, of which the amino acid
sequence is shown in Figure 2(b) (iii);

(b)   6H1 VH, of which the amino acid sequence is shown
in Figure 2(a) (i), and 6H1, of which the amino acid
sequence is shown in Figure 2(b) (i);

(c)   6H1 VH, of which the amino acid sequence is shown
in Figure 2(a) (i), and 6A5 VL, of which the amino acid
sequence is shown in Figure 2(b) (ii).

7.   A specific binding member according to claim 6
wherein said human antibody antigen binding domain
comprises the VH domain 6H1 VH, of which the amino acid
sequence is shown in Figure 2(a) (i), and the VL domain
6B1 VL, of which the amino acid sequence is shown in
Figure 2(b) (iii).

8.   A specific binding member according to claim 3
wherein said human antibody antigen binding domain
comprises a complementarity determining region (CDR)
with an amino acid sequence identified as a CDR in any
of the sequences shown in Figures 19 (i) to (iv).

9.   A specific binding member according to claim 8
wherein said human antibody antigen binding domain
comprises a VH domain which comprises a CDR3 with a
sequence shown as CDR3 in Figure 19 (i).

10.   A specific binding member according to claim 3

which competes for binding to TGF-β2 with a specific binding member according to claim 6.

11.   A specific binding member according to claim 10 which competes for binding to TGF-β2 with a specific binding member according to claim 7.

12.   A specific binding member according to claim 3 which binds the peptide TQHSRVLSLYNTIN.

13.   A specific binding member according to claim 3 which binds the active form of TGFβ2 but not the latent form.

14.   A specific binding member according to claim 3 wherein said human antibody antigen binding domain comprises a VH sequence of the DP50 germ line, or a re-arranged form thereof.

15.   A specific binding member according to claim 1 or claim 2 wherein said human antibody antigen binding domain is for the TGF-β isoform TGF-β1.

16.   A specific binding member according to claim 15 wherein said human antibody antigen binding domain comprises a VH domain which has the amino acid sequence shown in any of Figure 1(a) (i), Figure 1(a) (ii) and Figure 1(c) (i).

17. A specific binding member according to claim 15 or claim 16 wherein said human antibody antigen binding domain comprises a VL domain which has the amino acid sequence shown in any of Figures 1(b) (i), 1(b) (ii)

5   and 1(a) (iii).

18. A specific binding member according to claim 17 wherein said human antibody antigen binding domain comprises a pairing of a VH domain and a VL domain selected from:

10 (a)   1B2 VH, of which the amino acid sequence is shown in Figure 1(a) (i), and 7A3 VL, of which the amino acid sequence is shown in Figure 1(b) (i);

(b)   31G9 VH, of which the amino acid sequence is shown in Figure 1(a) (ii), and 31G9 VL, of which the

15 amino acid sequence is shown in Figure 1(a) (iii);

(c)   27C1 VH, of which the amino acid sequence is shown in Figure 1(c) (i), and 10A6 VL, of which the amino acid sequence is shown in Figure 1(b) (ii).

19. A specific binding member according to claim 18

20 wherein said human antibody antigen binding domain comprises the VH domain 27C1 VH, of which the amino acid sequence is shown in Figure 1(c) (i), and the VL domain 10A6 VL, of which the amino acid sequence is shown in Figure 1(b) (ii).

25 20. A specific binding member according to claim 15

wherein said human antibody antigen binding domain comprises a VH domain which comprises a CDR3 with an amino acid sequence selected from those shown in Figure 3.

5   21.   A specific binding member according to claim 20 wherein said CDR3 has the sequence shown for CDR3 of 27C1 VH.

22.   A specific binding member according to claim 15 wherein said human antibody antigen binding domain is

10   comprises the 31G9 VH domain of which the sequence is shown in Figure 1(a) (ii) and the CS37 VL of which the sequence is shown in Figure 14.

23.   A specific binding member according to claim 15 which competes for binding to TGF-β1 with a specific

15   binding member according to claim 18.

24.   A specific binding member according to claim 23 which competes for binding to TGF-β1 with a specific binding member according to claim 19.

25.   A specific binding member according to claim 15

20   which competes for binding to TGFβ1 with a specific binding member according to claim 22.

26.   A specific binding member according to claim 15

which binds the peptide TQYSKVLSLYNQHN.

27.   A specific binding member according to claim 1
wherein said human antibody antigen binding domain is
for the TGF-β isoforms TGF-β1 and TGF-β2.

5   28.   A specific binding member according to claim 27
wherein said human antibody antigen binding domain
comprise a VL domain with the amino acid sequence shown
in Figure 4 and a VH domain with the amino acid
sequence shown in Figure 1(a) (ii).

10   29.   A specific binding member according to claim 27
which competes for binding to TGF-β1 and for binding to
TGF-β2 with a specific binding member according to
claim 28.

30.   A specific binding member according to any
15   preceding claim comprising a single-chain Fv antibody
molecule.

31.   A specific binding member according to any of
claims 1 to 29 which comprises one or more amino acids
in addition to those forming said human antibody
20   antigen binding domain.

32.   A specific binding member according to claim 31
comprising an antibody constant region.

33.   A specific binding member according to claim 32 which comprises a whole antibody.

34.   A specific binding member according to claim 32 or 33 wherein said antibody constant region is IgG4
5   isotype.

35.   A method comprising causing or allowing binding of a specific binding member according to any preceding claim to TGF-β1 isoform and/or TGF-β2 isoform of human TGF-β.

10   36.   A method according to claim 35 wherein binding takes place *in vitro*.

37.   A method according to claim 35 wherein binding takes place *in vivo*.

38.   A method according to any of claims 35 to 37
15   wherein said binding of the specific binding member neutralises said isoform or isoforms.

39.   Use of a specific binding member according to any of claims 1 to 34 in the manufacture of a medicament for treating an individual to counteract effects of
20   TGF-β which are deleterious to the individual.

40.   Use according to claim 39 wherein said effects

are fibrosis promoting effects.

41.   Use according to claim 40 wherein said individual has a condition selected from the group consisting of glomerulonephritis, neural scarring, dermal scarring,

5   ocular scarring, lung fibrosis, arterial injury, proliferative retinopathy, retinal detachment, adult respiratory distress syndrome, liver cirrhosis, post myocardial infarction, post angioplasty restenosis, keloid scarring, scleroderma, vascular disorders,

10   cataract, and glaucoma.

42.   Use according to claim 41 wherein said condition is neural scarring or glomerulonephritis.

43.   Use according to claim 39 wherein said effects contribute to an immune or inflammatory disease

15   condition.

44.   Use according to claim 43 wherein said condition is selected from the group consisting of rheumatoid arthritis, macrophage deficiency disease and macrophage pathogen infection.

20   45.   Nucleic acid encoding a specific binding member according to any of claims 1 to 34.

46.   Nucleic acid according to claim 45 which is part

of an expression vector.

47.   A method which comprises use of nucleic acid according to claim 45 or claim 46 in an expression system for production of a specific binding member
5   according to any of claims 1 to 29.

48.   A host cell containing nucleic acid according to claim 45 or claim 46.

49.   A host cell according to claim 48 which is capable of producing said specific binding member under
10   appropriate culture conditions.

50.   A method of producing a specific binding member according to any of claims 1 to 34 comprising culturing a host cell according to claim 49 under appropriate conditions for production of said specific binding
15   member.

51.   A method according to claim 50 wherein following said production said specific binding member is isolated from the cell culture.

52.   A method according to claim 51 wherein following
20   said isolation the specific binding member is used in formulation of a composition comprising at least one additional component.

53.   A method according to claim 52 wherein said composition is a pharmaceutical composition comprising a pharmaceutically acceptable excipient.

54.   A pharmaceutical composition comprising a

5   specific binding member according to any of claims 1 to 34 and a pharmaceutically acceptable excipient.

55.   A method of treatment of a condition in which effects of TGF-β are deleterious to an individual, the method comprising administration of a pharmaceutical

10   composition according to claim 54 to the individual.

56.   A method according to claim 50 wherein said effects are fibrosis promoting effects.

57.   A method according to claim 56 wherein said individual has a condition selected from the group

15   consisting of glomerulonephritis, neural scarring, dermal scarring, ocular scarring, lung fibrosis, arterial injury, proliferative retinopathy, retinal detachment, adult respiratory distress syndrome, liver cirrhosis, post myocardial infarction, post angioplasty

20   restenosis, keloid scarring, scleroderma, vascular disorders, cataract, and glaucoma.

58.   A method according to claim 57 wherein said condition is neural scarring or glomerulonephritis.

59.   A method according to claim 55 wherein said effects contribute to an immune or inflammatory disease condition.

60.   A method according to claim 59 wherein said condition is selected from the group consisting of rheumatoid arthritis, macrophage deficiency disease and macrophage pathogen infection.

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Claims searched:	1 to 60	Date of search:	20 January 1997

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Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

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Int Cl (Ed.6): CO7K 16/22

Other: ONLINE: WPI, CABS, EMBASE, CEABA, DBA, CBA

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
Y	EP 0290012 A1	(ONCOGEN) see page 4 lines 55 to 58 Claims 15 to 17.	Claim 1 at least
Y	WO 95/26203 A1	(UNIVERSITY OF MANCHESTER) see page 1 lines 1 to 13, page 4 line 18 to page 5 line 3 and the example.	Claim 1 at least
Y	WO 93/11236 A1	(MRC & CAMBRIDGE ANTIBODY TECHNOLOGY) see page 1 line 3 to page 2 line 11 and page 27 lines 11 to 25.	Claim 1 at least
Y	Nature, Vol. 346, 26th July 1990, Border *et al*, "*Suppression of experimental glomerulonephritis by antiserum against transforming growth factor $\beta 1$*", pages 371 to 374		Claim 1 at least

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

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